ejpmr, 2016,3(11), 128-137

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTI-INFLAMMATORY EFFECT, ANTIOXIDANT POTENTIALS AND PHYTOCHEMICAL INVESTIGATION OF *CRATAEGUS SINAICA* BOISS. ROOTS GROWING IN EGYPT.

[1]Atef A. El-Hela, *[1, 2]Nevein M. Abdelhady, [3]Hala M. El-Hefnawy, [4]Taghreed A. Ibrahim and [5]Gamil M. Abdallah

[1]Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt,
[2]Pharmacognosy Department, Faculty of Pharmacy, Deraya University, El-Minya, Egypt,
[3]Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Egypt,
[3,4]Pharmacognosy Department, Faculty of Pharmacy, King Saud University, Riyadh, KSA and
[5]Biochemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Corresponding Author: Dr. Nevein M. Abdelhady

Pharmacognosy Department, Faculty of Pharmacy, Deraya University, El-Minya, Egypt

Article Received on 07/09/2016 **Article Revised on 27/09/2016** **Article Accepted on 17/10/2016**

ABSTRACT

In vivo antioxidant and anti-inflammatory studies of the aqueous and methanol extracts, as well as, methylene chloride and ethyl acetate fractions of *Crataegus sinaica* Boiss. roots (Rosaceae) growing in Egypt were carried out. Anti-inflammatory effect was evaluated in rats using paw oedema ,which revealed that the methylene chloride fraction was the most potent followed by methanol extract, ethyl acetate fraction and aqueous extract respectively, meanwhile, the antioxidant potentials was performed through evaluation of their impact against Streptozotocin induced oxidative stress in liver and kidney tissues of albino rats revealed that the ethyl acetate fraction was the most potent followed by methanol and aqueous extracts, then methylene chloride fraction respectively. Phytochemical investigation of the methylene chloride and ethyl acetate fractions exhibiting the most significant anti-inflammatory and antioxidant effects resulted in isolation of four compounds, namely, cholesterol, ß-sitosterol, uvaol and oleanolic acid, and five compounds, namely, epicatechin, quercetin, hyperoside, rutin and vitexin respectively, the structures of the isolated compounds were elucidated on basis of their chromatographic and spectroscopic data.

KEYWORDS: *Crataegus sinaica* Boiss., Egypt, Roots, Triterpenes, Flavonoids, Anti-inflammatory and Antioxidant.

INTRODUCTION

Natural products became one of the most important resources for developing new compounds by virtue of their unique chemical diversity leading to versatile biological properties; they play a pivotal role in many drug development and research programs (**Yuan *et al.*, 2016 and El-Hela *et al.*, 2013).**

Genus *Crataegus* (Hawthorn) belongs to family Rosaceae; it includes about 280 species of deciduous spiny shrubs and small trees where most of these species are grown as ornamentals and hedging plants (**Hyam and Pankhurst 1995**), they are widely distributed in Northern temperate zones of Asia, Europe and America (**Leung and Foster 1996).**

Crataegus sinaica Boiss. is known in common as Za`roor Al-Awdya or Za`roor grows wildly on the mountains of Saint Catherine Protectorate, South Sinai, Egypt flowers in May to June bearing orange to red fruits **(Al-Barouki and Peterson 2007; Shahat *et al.*, 1996 and Tackholm 1974)**.

Many *Crataegus* species have been used in folk medicine since ancient time for the treatment of heart diseases as deficiency of the coronary supply and arrhythmias (**Fugh-Berman 2000)** due to their evident improvement of heart functions in declining cardiac performance equivalent to stage I and II in NYHA classification **(Refaat *et al.*, 2010; Ahmed *et al.*, 2001 and Amoun *et al.*, 1994 "part I")**, in addition to their reported antimicrobial, anti HIV (**Shahat *et al.*, 1998; Shahat *et al.*, 1996),** hepatoprotective **(Refaat *et al.*, 2010)**, cytotoxic **(Hamahameen and Jamal 2013),** hypotensive, antioxidant **(Amel *et al.*, 2014),** anti-obesity, antihyperglycaemic (**Al-Hallaq *et al.*, 2013),** antispasmodic, hypotensive and anti-atherosclerotic **(Ercisli *et al.*, 2015; Tahirović and Bašić 2014, Kumar *et al.*, 2012)**.

These plants are generally rich in proanthocyanidins, flavonoids and catechins which are the main constituents responsible their biological activities, moreover, they are used for quality control and standardization of their drug material and preparations (**Bahroun *et al.*, 1996; Schussler *et al.*, 1995)**.

Several researches concerning *C. sinaica*, reported the isolation of procyanidins, proanthocyanidins, flavonoids, epicatechin **(Nabavi *et al.*, 2015; Liu et al., 2011; Refaat *et al.*, 2010; Shahat *et al.*, 1998)** and ursolic acid (**Shahat *et al.*, 2002; Amoun *et al.*, 1994 "part II" and Amoun *et al.*, 1994 "part III")** from the leaves and fruits while, several publications concerned quantification of flavonoids and epicatechin in certain *Crataegus* species as *C. monogyna* Jacq., *C. pinnatifida* BGE and C. *microphylla* Koch (**Mateos *et al.*, 2012; Melikoglu *et al.*, 2004; Wittig *et al.*, 2002).** It worth noting that nothing was reported aboutthe biological and chemical investigation the roots of *C. sinaica* Boiss., so this study targeted biologically guided investigation of their chemical constituents.

MATERIAL AND METHODS

Plant material

Shrubs of *Crataegus sinaica* Boiss. roots were collected from Saint Catherine (Wadi Gebal) in South Sinai, Egypt during their flowering and fruiting stage (September 2014), their identities were established by Prof. Dr. Abdo Marey, Prof. of botany, Faculty of Science, Al-Azhar University. A voucher specimen (C.S. # 0905) was deposited in a herbarium in department of Pharmacognosy, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt. The plant roots were separated, air-dried, powdered (2mm mesh) and kept in tightly closed amber coloured glass containers protected from light at low temperature.

Standards

Cholesterol, β-sitosterol, uvaol and oleanolic acid, caffeic acid, gallic acid,catechin, epicatechin, quercetin, kamferol,hyperoside, rutin, isoorientin, orientin, isovitexin and vitexin were supplied by Department of Pharmacognosy, Medical University of Gdansk, Poland, while Epicatechin and Rutin were purchased from Sigma Company (St. Louis MO, USA).

Material for chromatographic analysis

TLC analysis was performed on pre-coated plates of silica gel F_{254} (E. Merck, Darmstadt, Germany), meanwhile detection was carried out through examination in UV light before and after exposure to ammonia vapors and spraying with 1% vanillin/ H_2SO_4 reagent. CC analysis was performed using Silica gel (60-120 mesh E. Merck, Darmstadt, Germany)), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), methanol, hexane, petroleum ether, ethyl acetate, methylene chloride (Al-Gamhoria Co. for Chemicals, Egypt).

Material for biological studies

Animals

Adult albino mice weighing (20-25g) and rats (150-200g) of either sex were purchased from The Animal House Laboratory, National Research Center, Cairo, Egypt, they were housed in an environmentally control room, maintained at uniform light and temperature conditions of and provided with food and water *ad libitum.*

Drugs

Vitamin C was obtained from PHARCO Company for pharmaceutical industries Alexandria, Egypt, Carrageenan was obtained from BDH Chemicals; Poole, England, Indomethacin was from EPICO Company for Pharmaceutical Industries, Cairo, Egypt, Glibenclamide (Daonil®) from Hochest Company for Pharmaceuticals, Cairo, Egypt, while Streptozotocin (Sigma Chemical Company, Saint Louis, MO, USA).

Diagnostic Kits

Pre-made kits for evaluation of blood glucose level (BGL), superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) in RBC_S and evaluation of serum ascorbic acid (Randox Laboratories, Crumlin, England) suitable for automatic biochemical analyzers.

Preparation of extracts

500 and 50 g finely-powdered sample were extracted separately in soxhlet systems with 2000 ml of 80% (v/v) aqueous methanol and 250 ml distilled water for one hour to yield the methanol and aqueous extracts respectively, they were filtered under vacuum through Whatmann No.1 filter paper, the residue was re-extracted following the same procedure two more times, extracts collected were vacuum dried at 40 ^{o}C to give 62 g and 7 g methanol and aqueous extracts respectively.

50 g of the methanol extract were suspended in water and successively fractionated using hexane, methylene chloride and ethyl acetate, each fraction was concentrated under reduced pressure to give 12 g, 8 g and 15 g respectively.

Biological Studies

a. Effect of the tested plant extracts & fractions in carrageenan induced inflammation

The anti-inflammatory effect of plant extracts and fractions was determined using the method of Winter **(Winter *et al.*, 1962)**; where rats were divided into seven groups, six animals each which were classified as follows; **group 1;** negative control (inflammatory control untreated), **group2;** inflammatory control treated with Indomethacin (10 mg kg^{-1} b.wt., **Pandurangana *et al.*, 2008**) , **group 3;** inflammatory control administered the aqueous extract (50 mg kg^{-1} b.wt.), **group 4;** Inflammatory control administered the methanol extract(50 mg kg^{-1} b.wt.), **group 5;** inflammatory control administered the methylene chloride fraction(50 mg kg^{-1}

b.wt.), **group 6;** inflammatory control administered the ethyl acetate fraction (50 mg kg^{-1} b.wt.) as well as **group 7** which is formed of normal untreated control group received standard saline solution (10 ml kg^{-1} b.wt.).

Paw edema was induced by subcutaneous injection of 0.1 ml of freshly prepared 1% carrageenan (in 0.3% CMC) suspension into the subplantar region of left hind paw of each rat, after administration of carrageenan, animals were deprived of water during experiments to ensure uniform hydration and to minimize variability in edematous response, the measurement of the paw volume was done on the principle of volume displacement using plethysmometer (21025 Comerio AV, Italy). The readings were taken before and at 0, 30, 60, 120 and 180 min intervals after the administration of carrageenan injection for a period of 3 hours. The edema at each time was calculated in relation to the paw volume before the injection of the carrageenan. The anti-inflammatory activity of the tested extracts and fractions were determined as the percentage of inhibition of inflammation after it was induced by carrageenan by taking volume of inflammation in inflammatory control groups.

The percentage inhibition was calculated by using the formula:
% Inhibition = [(Mean paw inflammation of inflammatory control – Mean paw inflammation of test)/ Mean paw inflammation of inflammatory control] ×100

b. Evaluation of antioxidant potentials
Induction of diabetes; rats were rendered diabetic by a single intraperitonial dose of freshly prepared Streptozotocin 45mg/kg body weight dissolved in saline where diabetes was identified in rats by moderate polydipsia and marked polyuria, 48 h., later fasting blood glucose levels were estimated, rats with blood glucose levels ranging between 200-350 mg/dl were considered diabetic and included in the experiment.

Experimental design; seventy diabetic rats were divided into seven groups- ten animals each (1:1 for males: females) – where divided into groups as follows; **group1**(negative control); received standard saline solution (10 ml kg-1 b.wt.), **group 2** (positive control); received the reference standard Glibenclamide (0.025 g kg-1 b.wt., **Abdelhady *et al.*, 2014**), **group 3**; received aqueous extract (50 mg kg^{-1} b.wt.), **group 4**; received methanol extract(50 mg kg^{-1} b.wt.), **group 5**; received methylene chloride fraction(50 mg kg^{-1} b.wt.), **group 6**; received ethyl acetate fraction (50 mg kg^{-1} b.wt.), as well as **group 7** which formed of normal untreated control group received standard saline solution (10 ml kg^{-1} b.wt.).

All the investigated extracts and fractions were given as single daily oral doses where they were sacrificed on 30th day by cervical dislocation, liver and kidney tissues′ homogenates were used for estimation of enzymatic antioxidants such as superoxide dismutase **(Williams *et al.*, 1983)** and catalase (**Cohen *et al.*, 1970)** and non-enzymatic antioxidants such as vitamin C **(Loh and Wilson 1973)** and glutathione **(Ellman 1959)** using Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY)**.**

Statistical analysis: The statistical analysis of the outcome data was carried out using one way analysis of variance (ANOVA) followed by student t-test, P value <0.05 were considered as significant **(Elliott and Woodward, 2007).**

Separation of compounds from methylene chloride and ethyl acetate fractions
The methylene chloride fraction (7g) was fractionated by column chromatography on silica gel (150g, 5 x 120cm) using a step gradient of petroleum ether- ethyl acetate (5 % to 20% ethyl acetate), fractions of 50 ml were collected and monitored by TLC. The identical elutes were pooled together to give 5 fractions, as follows; A (0.35g), B (1.4g), C (2.7g) and D (2.5g) respectively, from fractions A and B compounds 1 (100mg) and compound 2 (150mg) were obtained by preparative TLC, the developing solvent system was (chloroform: methanol, 9:1) followed by chromatography on Sephadex LH- 20 (10g, chloroform: methanol 1:1) while compound 3 (150 mg) was isolated from fraction C using silica gel column (10g ,10x0.5mm) and re-chromatography on Sephadex LH 20 (5g , 20x1mm) and chloroform: methanol as eluent meanwhile, compound 4 (84 mg) was obtained from fraction D through the same procedure.

The ethyl acetate fraction (10 g) was applied onto silica gel column (200g, 5x120 cm) and eluted with chloroform withincreasing amount of methanol 5-100% to give three fractions, where the elutes (50ml each) were collected and monitored by TLC using methylene chloride: methanol (85:15) as solvent system, the gained chromatograms were examined under UV light at 365nm before and after exposure to ammonia vapor where similar fractions were pooled and the solvents were separately evaporated under pressure to give three pooled fractions, they were as follows;fraction I (3g) was further purified on sephadex LH-20 to give compound 5 (120mg), fraction II (0.3g) was chromatographed on sephadex LH-20 using methanol to give compound 6 (10mg) and fraction III (2g) was purified by column chromatography on cellulose (60g, 3.5 x 120cm) using 10% methanol in water. From fraction 1, 10ml were collected and monitored by TLC using as solvent system to give 2 subfractions, they were as follows; sub-fraction 1 which was further purified using sephadex LH-20 to give compounds 7 (20mg) and compound 8 (30mg) and sub-fraction 2 (0.6g) which was also further purified to give compound 9 (100mg). UV spectral analysis for the isolated compounds were recorded on Unicam SP 1750 Pye spectrophotometer while ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-300 MSL

instrument (Bruker, Karlsruhe) using TMS as internal standard and EI-MS was obtained on Joel 100 (70 eV).

RESULTS AND DISCUSSION

Oxidative stress, defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses **(Baş *et al.*, 2015),** increased ROS production exerts toxic effects on membrane polyansaturated phospholipids, resulting in generation of toxic products **(Pandir *et al.*, 2016).** Diabetes, the most common endocrine disorder, characterized by increased blood glucose levels resulting from defective insulin secretion, its complications are associated with increased oxidative stress **(Amin *et al.*, 2015),** similarly, in the streptozotocin-induced diabetes, ROS levels increase, meanwhile antioxidant defense system is significantly altered **(Apaydın *et al.*, 2016)**. Long term, diabetes leads to complications as accelerated atherosclerosis, renal failure, inflammatory diseases, cardiovascular diseases, cataract which results from alteration in structural functions of collagen basement membrane and tissue damage **(Ganesh *et al.*, 2012).**

Screening of the antioxidant potentials of different extracts and fractions of *Crataegus sinaica* Boiss. roots on both the liver and kidney tissues of adult diabetic albino rats revealed that the most significant results were recorded for the ethyl acetate fraction which restored the decreased activities of SOD and CAT significantly caused by diabetes induced oxidative stress which was manifested by inadequacy of the antioxidant defenses, the most potent was the ethyl acetate fraction, methanol and aqueous extract respectively they produced significant increase in these enzymes by virtue of helping to scavenge free radicals compared to diabetic rats and the standard hypoglycemic drug Glibenclamide, tables (1 and 2).

Table (1); Enzymatic and non-enzymatic antioxidant potentials of aqueous extract, methanol extract, methylene chloride and ethyl acetate fractions of *Crataegus sinaica* Boiss. roots (50 mg kg^{-1} b.wt.) and Glibenclamide (0.025 mg kg^{-1} b.wt.) on liver tissues of adult albino rats:

Antioxidant Parameters		Normal control	Negative control	GLB	Extracts			
					Aqueous	Methanol	Methylene chloride	Ethyl acetate
Enzymatic	**SOD** U/g tissue	6.913± 0.092	3.933± 0.132	6.214± 0.130	5.740± 0.060	6.092± 0.081	4.614± 0.018	6.145± 0.070
	CAT µ mole H_2o_2 utilized/min/ mg protein	57.920± 0.240	42.817± 0.137	53.46± 0.201	45.319± 0.130	50.016± 0.075	46.215± 0.140	52.307± 0.195
Non-enzymatic	**Vit. C** mg/g fresh tissue	1.830± 0.019	0.745± 0.015	1.516± 0.018	0.980± 0.017	1.380± 0.055	0.944± 0.019	1.483± 0.017
	GSH µg/mg protein	46.810± 1.150	28.416± 0.170	43.17± 0.173	25.475± 0.130	39.860± 0.145	31.310± 0.110	42.350± 0.182

The tabulated results representing the means ± standard error, n = 10

Diabetes result in significant decrease in vitamin C levels compared control rats where its level was significantly restored in liver and kidney tissues of treated groups compared to the standard drug Glibenclamide meanwhile, GSH has a multifaceted role in antioxidant defense; it is a direct free radical scavenger; diabetic oxidative stress decrease GSH level in liver and kidney tissues compared to control where significant elevation of GSH levels were observed in treated groups compared to the standard drug Glibenclamide, tables (1 and 2).

Inflammation is an orchestrated biological process, induced by microbial infection or tissue injury **(Garcı´a-Lafuente *et al.*, 2009)**, there are various components of an inflammatory reaction that can contribute to the associated symptoms and tissue injury where oedema, leukocyte infiltration, and granuloma formation represent such components of inflammation **(Bisht *et al.*, 2014).**

Different extracts and fractions of *Crataegus sinaica* Boiss. roots were evaluated for their anti-inflammatory effect using carrageenan induced rat paw oedema model, the gained results are compiled tables 3 and 4. The overall gained results revealed that methylene chloride fraction was the most significant followed by methanol and aqueous extracts respectively, they reduced the carrageenan induced oedema by 39.58 %, 37.95 and 35.96% respectively at 3 h, whereas the standard drug (Indomethacin) showed 41.49 % of inhibition as compared to the control group.

Table (2); Enzymatic and non-enzymatic antioxidant potentials of aqueous extract, methanol extract, methylene chloride and ethyl acetate fractions of *Crataegus sinaica* Boiss. roots (50 mg kg^{-1} b.wt.) and Glibenclamide (0.025 mg kg^{-1} b.wt.) on kidney tissues of adult albino rats:

	Antioxidant Parameters	Normal control	Negative control	GLB	Extracts			
					Aqueous	Methanol	Methylene chloride	Ethyl acetate
Enzymatic	**SOD** U/g tissue	2.273 ± 0.106	0.875 ± 0.042	1.937 ± 0.041	1.925 ± 0.055	2.195 ± 0.128	1.420 ± 0.047	2.217 ± 0.145
	CAT µ mole H_2o_2 utilized/min/ mg	28.612 ± 0.090	14.900 ± 0.191	27.130 ± 0.258	22.460 ± 0.095	25.850 ± 0.110	18.835 ± 0.078	26.30 ± 0.105
Non-enzymatic	**Vit. C** mg/g fresh tissue	1.305 ± 0.021	0.485 ± 0.023	1.655 ± 0.030	1.464 ± 0.104	1.165 ± 0.032	0.940 ± 0.017	1.266 ± 0.040
	GSH µg/mg protein	47.495 ± 0.301	23.510 ± 0.195	43.360 ± 0.165	42.750 ± 0.180	43.980 ± 0.150	35.490 ± 0.214	45.350 ± 0.137

The tabulated results representing the means ± standard error, n = 10

Table (3): Percent oedema inhibition in carrageenan induced inflammation of aqueous extract, methanol extract, methylene chloride and ethyl acetate fractions of *Crataegus sinaica* Boiss. roots (100 mg kg^{-1} b.wt.) of adult albino rats

Time (min)	Normal control	Negative control	Positive control (Indomethacin)	Extracts			
				Aqueous	Methanol	Methylene chloride	Ethyl acetate
0	---	---	6.35	1.33	2.60	3.26	1.25
30	---	---	31.20	25.50	27.19	28.74	22.41
60	---	---	36.56	31.14	33.27	34.95	27.85
120	---	---	45.65	40.85	41.68	43.24	38.70
180	---	---	41.49	35.96	37.95	39.58	32.10

Fractionation and purification of both methylene chloride and ethyl acetate fractions was carried out being the most potent biologically as anti-inflammatory and antioxidant respectively where four compounds were isolated from methylene chloride fraction, they all gave positive Liebermann's Burchard test **(Harborne 1998),** the data gained was as follows;

Compound 1(100 mg): colorless crystals (MeOH), mp 148˚-150˚C, R_f 0.81 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 386 (M^+, $C_{27}H_{46}O$, 5); 368 (M^+- H_2O, 20); 353 (M^+- (H_2O- Me); 311 (15) ; 258 (100); 243 (9.9), 217(7) ;151 (5), 135 (5),109 (17), 95 (22), 81(23) and 55 (65).

Compound 2 (150mg): white crystals (MeOH), mp 138˚-148˚C, R_f 0.77 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 414 (M^+, $C_{29}H_{50}O$, 100); 396 (M^+- H_2O,50) , 353 (M^+- (H_2O- Me), 255 (M^+ - side chain, 20),243 (9.9); 217(7) ;151 (5), 135 (5),109 (17), 95 (22), 81(23) and 55 (65).

Table (4): Anti-inflammatory activity of aqueous extract, methanol extract, methylene chloride and ethyl acetate fractions of *Crataegus sinaica* Boiss. roots (100 mg kg^{-1} b.wt.) of adult albino rats:

Time (min)	Normal control	Negative control	Positive control (Indomethacin)	Extracts			
				Aqueous	Methanol	Methylene chloride	Ethyl acetate
0	1.492±0.041	1.593±0.029	1.492±0.115	1.533±0.070	1.518±0.087	1.501±0.092	1.562±0.091
30	1.455±0.040	3.214±0.032	2.180±0.116	2.360±0.110	2.240±0.090	2.115±0.014	2.930±0.82
60	1.148±0.037	3.415±0.042	2.194±0.109	2.623±0.096	2.527±0.102	2.230±0.105	2.805±0.065
120	1.490±0.033	4.072±0.019	2.205±0.110	3.118±0.114	2.630±0.107	2.330±0.108	3.514±0.048
180	1.433±0.030	3.688±0.038	2.219±0.120	2.840±0.115	2.317±0.065	2.295±0.109	3.107±0.130

The tabulated results representing the means ± standard error, n =

Compound 3 (150 mg): colorless prisms (MeOH), mp 222˚-225˚C, R_f 0.57 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 442 (M^+, $C_{27}H_{46}O$, 3); 427 (M^+- CH_3, 5); 393 (3), 234 (25), 207 (10%), 203(100%), 189 (9%), 175 (7%), 133 (10%).

Compound 4 (84 mg): white needle crystals (MeOH), mp 303˚ -305˚C, R_f 0.45 ($CHCl_3$-MeOH, 9:1). EI-MS *m/z* (% rel.int.): 456 (M^+, $C_{15}H_{10}O_7$, 5), 248 (100%), 219 (4), 203 (12), 133 (10).

The mass spectrum of compound 1 showed molecular ion peak at 386 m/z, which is likely for molecular formula $C_{27}H_{46}O$, was close to the published data for cholesterol **(Chang *et al.*, 2015; Horn and Chapman 2012),** the mass spectrum of compound 2 showed a molecular ion peak of 414 m/z ($C_{29}H_{50}O$), this mass values along with fragmentation pattern were found in conformity with those published for *β*-sitosterol in the literature (**Kamboj and Saluja 2011),** while the mass spectra of compounds 3 and 4 showed molecular ion peaks at m/z 442 and 456 , which are likely for molecular formula $C_{30}H_{50}O_2$ and $C_{30}H_{48}O_3$, both the mass spectra and fragmentation patterns were identical with the published data on uvaol (**Hatem and Najah 2016)** and oleanolic acid (**Gohari *et al.*, 2009).**

Further confirmation for the previous compounds was carried out by direct comparison with Co-chromatography versus their authentic samples, determination of melting point and mixed melting point.

Fractionation of the ethyl acetate fraction achieved the isolation and characterization of the following compounds:

Compound 5 (190 mg): pale yellow amorphous powder, R_f 0.48 ($CHCl_3$-MeOH: H_2O, 80:20:2). UV λ_{max} nm (MeOH): showed maxima 280nm. EI-MS *m/z* (% rel.int.): 290(54), 152 (47), 139 (100), 123(39) 109 (14), 98(12), 107(9.2), 77(14), 69 (27) and 41(25). *IR spectra:* IR spectra [υmax (KBr)] showed band at 2600-3400 (broad), 1620, 1520, 1470, 1380, 1280, 1240, 1150, 1120, 1080, 1020, 820 cm-1.

^{1}H-NMR (500 MHz, DMSO): 1H-NMR spectra showed peaks at δ*TMS* 4.56 [H-2, d, J(H-2, H-3a) 7.8 Hz], 4.10 [H-3, ddd, J(H-3a, H-4e) 5.57 Hz, J(H-3a, H-4a) 8.53 Hz, J(H-3a, H-2a) 7.80Hz], 2.55 [H-4a, dd, J(H-4a, H-3a) 8.52 Hz, J(H-4a, H-4e) 16.10 Hz], 2.90 [H-4e, dd, J(H-4e, H-3a) 5.50 Hz, J(H-4e, H-4a) 16.10 Hz], 5.87 [H-6, d, J(H-6, H-8) 2.3 Hz], 6.01 [H-8, d, J(H-8, H-6) 2.3Hz], 6.89 [H-2′, d, J(H-2′, H-6′) 1.95 Hz], 6.79 [H-5′, d, J(H-5′, H-6′) 8.07 Hz], 6.73 [H-6′, dd, J(H-6′, H-2′) 1.94 Hz, J(H-6′, H-5′) 8.19 Hz] and 8.00 (phenolic protons, m). *^{13}C-NMR (300 MHz DMSO):* Carbon atoms showed peaks at *δTMS* 27.7 (C-4), 66.3 (C-3), 81.0 (C-2), 93.8 (C-6), 95.3 (C-8), 114.54 (C-2), 115.13 (C-5), 18.44 (C-6) and other aromatic carbons showed peaks at *δ* of 99.3, 130.8, 144.7, 144.6, 155.3, 156.3 and 156.5.

Compound 5 is the major compound in the ethyl acetate extract, the mass spectrum of compound 5 showed a molecular ion peak at m/z 290, which is likely for $C_{15}H_{14}O_6$, the base peak at 139 m/z is characteristic for flavane nucleus confirmed by the presence of a single band in UV at 280nm **(Harborne 1998).** Further investigation of NMR spectra of this compound showed the presence of aliphatic protons at 4.7, 4.1 and carbons at 81 and 67 ppm confirm the presence of a flavan structure. In addition to phenolic protons and carbons suggest that this compound is a flavan with 5,7,3,4, tetrahydroxy flavan 3-ol (epicatechin), the structure was confirmed by direct comparison with authentic sample and previous published data **(Shahat *et al.*, 1998).**

Compound 6 (10 mg): yellow crystals (MeOH), mp 298-300˚C, Rf 0.52 (CHCl3-MeOH, 9:1). UV λmax (MeOH): 255,269 nm, EI-MS m/z (% rel.int.): 302 (M+, C15H10O7, 100); *^{1}H-NMR* (500 MHz, DMSO): δ 7.72 (1H, d, J = 2.5 Hz, H-2'), 7.60 (1H, dd, J = 8.5 & 2.5 Hz, H-6`), 6.85 (1H, d, J = 8.5 Hz, H-5'), 6.35 (1H, d, J = 2.5 Hz, H-6) and 6.15 (1H, d, J = 2.5 Hz, H-8); *^{13}C-NMR* (DMSO-d6): δ 177.2 (C- 4), 165.2 (C-7), 162.5 (C-5), 158.4 (C-2), 149.2 (C-9), 148.1 (C- 4'), 146.1 (C-3'), 138.3 (C-3), 124.3 (C-6'), 121.6 (C-1'), 116.2 (C-2'), 115.2 (C-5'), 104.6 (C-10), 99.2 (C-6) and 94.4 (C-8); MS: m/z 302, 281, 273, 207, 95, 81.

Compound 6 is soluble in mixture of chloroform and methanol with mp 312^{o}C , it gave negative Molisch's test and positive magnesium in concentrated hydrochloric acid test, it has yellow colour under UV 365 nm changed to intensive yellow after spraying with 2 % aluminum chloride reagent this behavior indicates that it is of flavonoid aglycone nature, EI-MS spectrum showed a molecular ion peak at m/z 302 while UV spectral data, showed large bathochromic shift in band I with aluminum chloride reagent which disappear on addition of hydrochloric acid that is an evidence of orthodihydroxy B ring (**Mabry *et al*, 1970**).

^{1}H-NMR spectrum showed an ABX coupling system of three proton signals as meta doublet (H-2`), ortho-meta dd (H-6`) and ortho doublet (H-5`) for 3`, 4` dihydroxy B-ring and AM-spin coupling system of two meta coupled protons at 6.38, 6.18 assigned H-8 and H-6 respectively while ^{13}C-NMR spectral data δppm exhibited fifteen typical carbon resonances for quercetin moiety were assigned in the aromatic region, showed two key signals of quercetin aglycone were assigned at 149.15 (C-4`) and 145.49 (C-3`) ppm, accordingly, compound **6** was identified as ***3, 5, 7, 3`, 4`, pentahydroxy –flavone (quercetin)*** (**Agrawal 1989 and Harborne *et al*, 1975**).

Compound 7 (20 mg): yellow crystals (MeOH) m.p. 217- 219 ^{o}C. EIMS m/z (% rel.int.) 358 (0.9), 316 (1.1), 302 (100), 286 (4.7), 274 (4.6), 257 (1.9), 245 (3.3), 229 (3.5), 153 (5.2), 144 (1.9), 137 (5.9), 60 (8.0). *^{1}H-NMR* (500 MHz, DMSO): [δ 6.5 (1H, d, J = 2 Hz, H-8) and

6.3 (1H, d, J = 2 Hz, H-6)] and 3 proton signals of a B ring at δ 7.98 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.5 Hz, H-5') and 6.99 (1H, d, J = 8.5 Hz, H-2'). *^{13}C-NMR (300 MHz DMSO):* (C2)159,1, (C3) 136.6, (C4) 180.2, (C5) 163.7, (C6)100.7,(C7)167.1,(C8)95,6, (C9) 159, (C10)106.3, (C1') 123.1(C2') 169.9, (C3') 146.3,(C4') 150.2(C5')118.3 (C6')132.7, (C1'') 106.3, (C2'') 73.4, (C3'') 75.8, (C4'')70.8, (C5'') 78.8, (C6'')62.8.

FAB-MS analysis of compound 7 exhibited a molecular weight FAB$^+$ of 465,the UV spectrum in MeOH , indicated the possible flavonol skeleton The ^{1}H-NMR (500 MHz, MeOD) spectrum of compound 7showed the presence of quercetin as aglycone including 2 proton signals of an A ring [δ 6.5 (1H, d, J = 2 Hz, H-8) and 6.3 (1H, d, J = 2 Hz, H-6)] and 3 proton signals of a B ring at δ 7.98 (1H, d, J = 2.0 Hz, H-6'), 7.72 (1H, dd, J = 8.5, 2.5 Hz, H-5') and 6.99 (1H, d, J = 8.5 Hz, H-2'). The ^{1}H-NMR (500 MHz, MeOD), therefore, the aglycone of compounds 7 was assigned to quercetin, the coupling constants (J), signal splitting patterns and chemical shifts in the proton and carbon signals of the sugar moieties suggested that the sugars was galactose. Therefore, the compound was assigned as quercetin 3-O-β-D-galactopyranoside (hyperoside); that was confirmed with standard samples using Co-chromatography. ^{1}H-NMR and ^{13}C-NMR analyses of the hyperoside standard samples revealed similarities in the coupling constants (J), signal splitting patterns and chemical shifts in the proton and carbon signals with the isolated compounds. Based on UV-vis., MS, ^{1}H-NMR and ^{13}C-NMR analyses, the spectral characteristics of this compound was found to be identical with this in the literature (**Cho *et al.*, 2008; Lee *et al.*, 2011).**

Compound 8 (30 mg): Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 260, 366, λ_{max} (MeONa) nm: 273, 315, 412, λ_{max} (AlCl3) nm: 276, 305, 439, λ_{max} (AlCl3/HCl) nm: 275, 303, 409, λ_{max} (AcONa) nm: 277, 320, 395, λ_{max} (AcONa/boric acid) nm: 261, 389; *^{1}H NMR* (DMSO-d6, 500 MHz) δ12.5 (OH), 7.54 (1H, br s, H-2`), 7.52(1H, d, J=8.0 Hz, H-6`), 6.83 (1H, d, J=8.0 Hz, H-5`), 6.45 (1H, br s, H-8), 6.22 (1H, br s, H-6), 5.40 (1H, d, J=7.6 Hz, H-1``), 4.39 (1H, d, J=2.5 Hz, H-1```), 3.05- 3.38 (10H, m, H-2```-H-6`` of glc and H-2```-H-5``` of rha), 0.95 (1H, d, J=6.0 Hz, H-6``); *$^{13}$C NMR* (DMSO-d6, 125 MHz) δ177.74 (C, C-4), 164.72 (C, C-7), 161.49 (C, C-5), 157.17 (C, C-2),156.75 (C, C-9),148.87 (C, C-4`), 145.18 (C, C-3`), 133.68 (C, C-3), 122 (CH, C-6`), 121.58 (C, C-1`), 116.60(CH, C-5`), 115.76 (CH, C-2`), 105 (C, C-10), 101.57 (CH, C-1``), 101.16 (CH, C-1```), 99. 2 (CH, C-6), 94.14 (CH, C-8), 76.83 (CH, C-3``), 76.19 (CH, C-5``), 74.43 (CH, C-2``), 72.23 (CH, C-4```), 70.92 (CH, C-3```), 70.69 (CH, C-2```), 70.36 (CH, C-4``), 68.61 (CH, C-5```), 67.40 (CH2, C-6``), 18.11 (CH3, C-6```); ESIMS c 633 [M$^+$Na]$^+$.

Compound 8 showed that Ring B contained three protons, H-5` appeared separately as doublet up filed at 6.79 (J = 8.4 4Hz) due to the shielding effect of oxygen substitution while both H-2` and H-6`appeared as multiplet downfield at 7.53 due to the deshielding effect of ring C meanwhile, ring A contained two protons, each of them appeared as doublet at 6.22 assigned for H-8 and 6.1 assigned for H-6. The coupling constant J = 1.8 Hz in both protons indicating that they were meta-coupled. Additional signals of sugar moiety, at 5.24 (H-1`` with J1``* 2`` axial = 6.6 Hz) indicated anomeric glucose proton with B-linkage and this high value confirm 3-glycosidation rather 5 or 7-glycosidation), while those at 4.37 (H-1``` with J1```* 2``` euqatorial = 1.5 Hz) and at 0.99 (Three protons of methyl) revealed rhamnose with α- linkage, these data indicate rutinosyl moiety, **(Mabry *et al.*, 1970).**

^{13} C-NMR, showed 27 carbon atoms, 12 of them for signals representing carbon atoms of the rhamnoglucosly moiety at 101.81 (confirming O-glycosidation rather than C-glycosidation), 100.81,76.57, 75.87, 74.17, 71.95, 70.61, 70.39, 69.99, 68,29, 67.07 and 17.80 (of rhamnosyl moiety), while the rest 15 carbon atoms were assigned to quercetin nucleus presented as follows: 9 aromatic non-oxygenated carbons viz. (156.75, 121.62, 115.92, 115.28, 102.57, 99.7 and 94.18), 5 oxyaromatic carbons viz. (167.95, 161.05, 149.27, 145.10 and 133.01) and one carbonyl carbon at 176.71, **(Agrwal, 1989),** and so, compound 8 was identified as quercetin-3-O-α-L-rhamnosyl-β-glucopyranoside (Rutin), previously isolated from of fruits and leaves *C .sinaica* **(Shahat *et al.*, 2002).**

Compound 9 (100 mg): Amorphous yellowish powder, mp 250-252˚C, Rf 0.52 (CHCl3-MeOH, 9:1). EI-MS m/z270 (% rel.int.): (M$^+$, $C_{15}H_{10}O_7$, 100); UV λ_{max} (MeOH) nm: 267, 296 (sh), 336, λ_{max} (MeONa) nm: 275, 324, 392, λ_{max} (AlCl3) nm: 276, 301, 348, 384, λ_{max} (AlCl3/HCl) nm: 276, 299, 340,381, λ_{max} (AcONa) nm: 274, 301, 376, λ_{max} (AcONa/boric acid) nm: 268, 302, 338; *^{1}H NMR* (DMSO-d6, 500 MHz); 6.3 (d, J= 2.5 Hz, H-6), 6.45 (d, J =2.5 Hz, H-8), 6.59 (S, H-3), 6.92 (d, J =8Hz, H-3`, H-5`), 7.85(d, J =8Hz, H-2`, H-6`), **1``** 4.59, d, 9.8, **2``** 3.85, d, 9.6, **3``** 3.21, t, 8.4, **4``** 4.05, t, 9.1, **5``**3.15, m , **6``** 3.69, d, 11.3; ^{13}C NMR (DMSO-d6, 125 MHz): C2 (166.18), C3 (104.21), C4 (183.92), C5 (166.3), C6 (100.37), C7 (163.2), C8 (95.23), C9 (159.25), C10 (103.85), C1`(121.5), C2`(129.49), C3`(117.1), C4` (162.87), C5`(116.87), C6`(129.49), **1``** 73.5, **2``** 71, **3``** 79.4, **4``**70.6, **5``** 82, **6``**61.9; ESI/MS c 456 [M$^+$Na]$^+$.

Compound 9 was isolated as yellow amorphous powder, m.p. 246-250 ^{0}C, gave positive Molisch's test, with TLC investigation yielded one purple spot on TLC under lamp (365 nm) changed to intensive yellow colour after spraying with AlCl3 2% in methanol, while paper chromatography investigation yielded one spot R_f S_1=0.48 [4:1:5 BAW] and S_2 =0.53 [15% CH_3COOH in H_2O]. EI-MS showed a peak at m/z 414 [M-H_2O]+ and 269 [aglycone-H]+, this compatible to the molecular

formula $C_{21}H_{20}O_{10}$ after refluxed with (2N) methanolic HCl for ten hours give two spots suggested the C-glycosidic nature. 1H-and 13C- NMR spectra revealed the characteristic chemical shifts and coupling patterns for a 5,7, 4'-oxygenated flavone. In the 1H-NMR spectrum of (**Sary *et al.*, 2004)**, an AA`BB` spin-system appear as a doublet at δ 7.93 (2H d , J = 8.7 Hz, H-2', H-6') and 6.94 (2H d , J = 8.6 Hz, H-3', H-5') indicated that the ring- B was substituted at C-4', the signal at δ 6.78, appear as a singlet, integrated for one proton, was assigned to H-3. From the following evidence, the last aromatic proton at δ 6.55, (1H, s, H-8) founded to be singlet and absence of H-6. this indicate that H-6 substituted by the glucone moiety as C-glycoside which proved previously through mass and also not hydrolyzed under normal scheme for O-glycoside . The proton signal at δ 13.56 suggested a OH group at C-5. The 1H-NMR spectrum exhibited anomeric proton signal at δ 4.59 ppm (1H, d, J=9.8 Hz, H-1`` of glucose). The upfield shift of anomeric proton is another evidence for C-glucosidation. The 13C-NMR spectrum of revealed the characteristic assignment patterns for a flavone with C-ring at C-3 (δ103.22) and C-4 (δ182.41), A-ring functionality at C-5 (δ161.11) and C-7 (δ163.89), and B-ring at C-4` (δ161.69). The use of a combination of 13C and DEPT-NMR analysis indicated that exhibited 21 carbon resonances; 11 methines (6 for aglycone and 5 for glucose), methylene at C-6`` (δ 61.92) and by differences from broad band spectrum, 9 quaternary carbon atoms were observed. Acid hydrolysis yielded two spots for isovitexin with Rf S_1 =0.57, S_2 =0.54 and vitexin with Rf S_1 =0.42, S_2 =0.40 (Wessely Moser rearrangement) those was identical with standard. The identity of aglycone and sugar attached was confirmed by FeCl3 oxidative hydrolysis (Wessely Moser rearrangement) those spots one for aglycone were detected with Rf S1 =0.12, S2 =0.87 on PC, which identical for 4',5,7-trihydroxyflavone (apigenin) and the sugar part was identical to glucose stander. From the above data, hydrolysis and comparison with stander as well as by literature comparison (335,336), the structure of (**Lin *et al.*, 2000, elhela *et,al* 2007)**] was assigned as: apigenin-6-c-glucopyranoside (vitexin).

From these results, the chemical structure of this compound was identified as apigenin-8-C-β-glucopyranoside (vitexin), and was elucidated on previously reported data on its structure

REFERENCES

1. **Abdel-Hady N.M., Abdallah G.M. and Idris N.F. (2014):** "phytochemical studies and *in vivo* antioxidant activity of two *Lavandula* species (lamiaceae) against streptozotocin induced oxidative stress in albino rats", *J. Biomed. & Pharm. Res.*, 3 (4): 30-40.
2. **Agrawal P.K. (1989):** "^{13}C NMR of Flavonoids", Chapter 6, Elsevier, New York.
3. **Ahmed A.A., Awatef M.K., Mary H.G. and Malek M.S. (2001):** "A new eudesmanolide from *Crataegus flava* fruits", *Fitoterapia*, 72: 756-9.
4. **Al Barouki E. and Peterson A. (2007):** "Molecular and morphological characterization of *Crataegus* L. species (*Rosacae*) in Southern Syria", Botanical Journal of the Linnean Society, 153: 255-263.
5. **Al-Hallaq E.A., Kasabri V., Abdalla S.S., Bustanji Y.K., Fatma U. and Afifi F.U. (2013):** "Anti-obesity and Antihyperglycaemic Effects of *Crataegus aronia* Extracts: *In Vitro* and *in Vivo* Evaluations", *Food and Nutrition Sciences*, 4: 972-983.
6. **Amel B., Seddik K., Shtaywy A., Saliha D., Mussa A., Assia B., Abderahmane B. and Smain A. (2014):** "Phytochemical analysis, antioxidant activity and hypotensive effect of Algerian Azarole (*Crataegus azarolus* L.) leaves' extracts", *RJPBCS*, 5:2, 286- 305.
7. **Amin A.H, El-Missiry M.A. and Othman A.I. (2015):** "Melatonin ameliorates metabolic risk factors, modulates apoptotic proteins, and protects the rat heart against diabetes-induced apoptosis", *Eur J Pharmacol.*, 747: 166–173.
8. **Ammon H., Kaul R. and Wirkungen H.K. (1994):** "Von *Crataegus* extrakten Flavonoiden und Procyanidinen Teil 2: Historisches und Wirkstoffe", *Dtsch Apoth Ztg*, 134: 2521-35.
9. **Ammon H., Kaul R. and Wirkungen H.K. (1994):** "Von *Crataegus* extrakten flavonoiden und procyanidinen Teil 1, Historisches und Wirkstoffe", *Dtsch Apoth Ztg*, 134: 2433-6.
10. **Ammon H., Kaul R. and Wirkungen H.K. (1994):** "Von *Crataegus* extrakten flavonoiden und procyanidinen Teil 3: Historisches und Wirkstoffe", *Dtsch Apoth Ztg*, 134: 2631-6.
11. **Apaydın F.G., Baş H., Kalender S. and Kalender Y. (2016):** "Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats", *Environ Toxicol Pharmacol.* , 41: 219-224.
12. **Bahroun T., Gressier B., Trotin F., Brunet C., Dine T., Luyckx, M. Vasseur J., Cazin M., Cazin J.C. and Pinkas M. (1996):** "Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations", *Arzneimittelforschung*, 46(11): 1086-9.
13. **Baş H., Kalender Y., Pandır D. and Kalender S. (2015):** "Effects of lead nitrate and sodium selenite on DNA damage and oxidative stress in diabetic and non-diabetic rat erythrocytes and leucocytes", Environ Toxicol Pharmacol., 39: 1019-1026.
14. **Bisht R., Bhattacharya S. and Jaliwala Y.A. (2014):** "COX and LOX inhibitory potential of *Abroma augusta* and *Desmodium gangeticum*", *The Journal of Phytopharmacology*, 3(3): 168-175.
15. **Chang C.T., Yang C.Y., Tsai F.J., Lin S.Y. and Chen C.J.(2015):** "Mass spectrometry-based proteomic study makes high-density lipoprotein a biomarker for atherosclerotic vascular disease", *BioMed Research International*, 2015; 164846: 13.
16. **Cho J.Y., ji S.H., Moon J.H., Lee K.H., Jung K.H. and Park K.H. (2008):** " A novel benzoyl

glucoside and phenolic compounds from the leaves of *Camellia japonica*", *Food Sci. biotechnol.*, 17: 1060-1065.

17. **Cohen G.J., Dembiec D. and J. Marcus (1970):** "Measurement of catalase activity in tissue extracts", *Anal. Biochem*, 34, 30-38.
18. **El-Hela A. A., El-Hefnawy H.M., Ibrahim T.A and Sleem A.A. (2007):** "Phytochemical and biological study of *crataegus sinaica* boiss. growing in Egypt", *The 7 th international Saudi Pharmaceutical Conference***,**19-21 March 2007,(29 Safar- 2 Rabia-1-1428H), P-105.
19. **El-Hela A.A., Abdel-Hady N.M., Dawoud G.T.M., Hamed A.M. and Morsy T.A (2013):** "Phenolic content, antioxidant potential and *Aedes Aegyptii* ecological friend larvicidal activity of some selected Egyptian plants", *J. Egypt. Soc. Parasitol.*, 43(1): 215-234.
20. **Elliott A.C. and Woodward W.A. (2007):** "Statistical analysis quick reference guidebook: with SPSS examples", ISBN: 9781412925600.
21. **Ellman, G. (1959):** "Tissue sulfhydryl groups", *Archives of Biochemistry and Biophysics*, 32: 70-77.
22. **Ercisli S., Yanar M., Sengul M., Yildiz H., Topdas E,.F., Taskin T., Zengin Y., Ugurtan K. and Yilmaz S. (2015):** "Physico-chemical and biological activity of hawthorn (*Crataegus spp.* L.) fruits in Turkey, *Acta Sci. Pol., Hortorum Cultus* 14(1): 83-93.
23. **Fugh-Berman A. (2000):** "Herbs and dietary supplements in the prevention and treatment of cardiovascular diseases", *Pre. Cardiol*., 3(1): 24.
24. **Ganesh E., Chowdhury A., Malarvani T. and Vardhan N.A. (2012):** "Microscopical observation of kidney for ameliorative effects of vitamin – E & C in streptozotocin-induced diabetic rats", *International Journal of Life Science and Pharma Research*, 2: 3.
25. **Garcı´a-Lafuente A., Guillamo´n E., Villares A., Rostagno M.A., Martı´nez J.A. (2009):** "Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease", *Inflamm. Res.*, 58: 537–552.
26. **Gohari A.R., Saeidnia S., Hadjiakhoondi A., Abdoullahi M. and Nezafati M (2009):** "Isolation and quantitative analysis of oleanolic acid from *Satureja mutica* Fisch. & C. A. Mey.", *Journal of Medicinal Plants*, 8: 5.
27. **Hamahameen B. M. and Jamal B. (2013): "**Determination of flavonoids in the leaves of Hawthorn (*Crataegus Azarolus*) of Iraqi Kurdistan region by HPLC analysis", *International Journal of Bioscience, Biochemistry and Bioinformatics*, 3: 1: 67-70.
28. **Harborne J.B. (1998):** "Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis", 3rd Ed., Chapman and Hall: London. ISBN: 0-412-57270-2, 302.
29. **Harborne J.B., Mabry T.J. and Mabry H. (1975):** "The Flavonoids", Chapman and Hall, London.
30. **Hatem N.A. and Najah Z.M. (2016):** "Isolation and elucidation of some chemical constituents of *Lavandula officinalis*", *J. Chem. Pharm. Res.*, 8(3): 394-401.
31. **Horn P.J. and Chapman K.D. (2012)**: "Lipidomics in tissues, cells and subcellular compartments", *The Plant Journal*, 70: 69–80.
32. **Hyam R. and Pankhurst R. (1995)**: "Plants and their names", Concise Dictionary", 1st Ed., Oxford University, P. 132.
33. **Kamboj A. and Saluja A. K. (2011):** "Isolation Of stigmasterol and βsitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae)", *Int J Pharm Pharm Sci*, 3: 1, 9496.
34. **Kumar D., Arya V., Bhat Z.A., Khan N.A. and Prasad D.N. (2012):** "The genus *Crataegus*: chemical and pharmacological perspectives", *Braz. J. Pharmacogn.*, 1: 1-14.
35. **Lee H.H., Cho, J.Y., Moon J. H. and Park K.H. (2011):** "Isolation and Identification of Antioxidative phenolic acids and flavonoid glycosides from *Camellia japonica* flowers", *Hort. Environ. Biotechnol.*, 52: 270-277.
36. **Leung A.Y. and Foster S. (1996):** "Encyclopedia of Common Natural Ingredients Used in Food, Drugs and Cosmetics", 2nd Ed., New York: John Wiley.
37. **Lin Y. L., Kuo Y. H., Shiao M. S., Chen C. C. and Ou, J. C. (2000):** "Flavonoid glycosides from *Terminalia catappa* L.", *Journal of the Chinese Chemical society*, 47(1): 253-256.
38. **Liu P., Kallio H. and Yang B. (2011): "**Phenolic compounds in hawthorn (*Crataegus grayana*) fruits and leaves and changes during fruits ripening", *J. Agric. Food Chem.*, 59: 11141-11149.
39. **Loh H.S. and Wilson C.W.M. (1973):** "Relationship between leucocytes and plasma ascorbic acid concentrations", *British Medical journal*, 3:733.
40. **Mabry T.J., Markham K.R. and Thomas M.B. (1970):** "The Systematic Identification of Flavonoids", Springer Verlag; New York.
41. **Mateos R.G., Santelises L.A., Hernández M.S., Angel R.N. and Kite G. (2012):** "Total phenolic compounds, flavonoids and antioxidant activity in the flowers of *Crataegus Spp.* from México", *Agrociencia* 46: 651-662.
42. **Melikoglu G., Bitis L. and Melici A. (2004):** "Flavonoids of *Crataegus microphylla", Natural Product Research*, 18 (3): 211-213.
43. **Nabavi S.F., Habtemariam S., Ahmed T., Sureda A., Daglia M., Sánchez E.S. and Nabavi S.M. (2015):** "Polyphenolic composition of *Crataegus monogyna* Jacq.: From chemistry to medical applications", *Nutrients*, 7, 7708-7728.
44. **Pandir D., Unal B. and Bas H. (2016):** "Lycopene protects the diabetic rat kidney against oxidative stress-mediated oxidative damage induced by furan", *Braz. Arch. Biol. Technol.*, 59: e16150794.

45. **Pandurangana A., Khosa R.L. and Hemalathab S. (2008):** "Evaluation of anti-inflammatory and analgesic activity of root extract of *Solanum Trilobatum* Linn", *Iranian Journal of Pharmaceutical Research*, 7(3): 217-221
46. **Refaat A.T., Shahat A.A., Ehsan N.A., Yassin N., Hammouda F., Abou Tabl E. and Ismail S.I (2010):** "Phytochemical and biological activities of *Crataegus sinaica* growing in Egypt", *Asian Pacific Journal of Tropical Medicine*, 257-261.
47. **Sary, H. G., Ayoub, N. A., Singab, A. B., Ahmed, A. H., & Al-Azizi, M. M. (2004):** "Chemical constituents and molluscicidal activity of *Iris pseudacorus* L. cultivated in Egypt", *Bulletin Of Pharmaceutical Sciences-Assiut University*, 27(1): 161-170.
48. **Schussler, M., Holzl, J. and Fricke, U. (1995):** "Myocardial effects of flavonoids from *Crataegus* species", *Arzneim.Forsch.*, 45(8): 842-5.
49. **Shahat A., Ismail S., Hammouda F. (1998):** "Anti-HIV activity of flavonoids and proanthocyanidins from *Crataegus sinaica",* Phytomedicine, 5(2): 133-136.
50. **Shahat A.A., Hammouda F., Ismail S.I., Azzam, S.A., De Bruyne, T., Lasuvanpoel, B., Pieters, L. and Vlietinck, A. J. (2002):** "Antiviral and antioxidant activity of flavonoids and proanthocyanidins from *Crataegus sinaica*", *J. Planta Medica*, 68(8): 539- 41.
51. **Shahat A.A., Hammouda F., Ismail, S. I., Azzam, S.A., De Bruyne T., Lasuvanpoel B., Pieters L. and Vlietinck A.J. (1998):** "Flavonoids and Proanthocyanidins from *Crataegus sinaica"*, *Phytomedicine*, 5(2): 133- 136.
52. **Shahat A.A., Hammouda F., Ismail, S. I., Azzam, S.A., De Bruyne T., Lasuvanpoel B., Pieters L. and Vlietinck A.J. (1996):** "Anti-Complementary Activity of *Crataegus sinaica*, *Planta Medica*, 62: 10-3.
53. **Tackholm, V. (1974):** "Student's Flora of Egypt" 2nd Ed., Cairo University Cooperative Printing Co., Biuret. .
54. **Tahirović A. and Bašić N. (2014):** "Phenolic Content and Antioxidant Activity of *Crataegus Monogyna* L. Fruit Extracts", *Works of the Faculty of Forestry University of Sarajevo*, 2, 29-40.
55. **Williams J.A., Wiener G., Anderson P.H. and Mc-Murray C.H. (1983):** "Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep", *Res Vet Sci*, 34: 253-256.
56. **Winter C.A., Risley E.A. and Nuss G.W. (1962):** "Carrageenan induced edema in hind paw of the rats as an assay for anti-inflammatory drugs", *Proc. Soc. Exp. Biol. Med.*, 111: 544-547.
57. **Wittig, J., Leopolz I., Graefe E., Jaki B., Treuter D. and Veit M. (2002):** "Quantification of procyanidin in oral herbal medicnal products containing extracts of *Crataegus* species", *Arzneim.-Forsch./ Drug Res.*, 52(2): 89-96.
58. **Yuan, H., Ma Q., Ye L. and Piao G. (2016):** "The traditional medicine and modern medicine from natural products", *Molecules*, 21: 559.