Determination of Flavonoids in the Leaves of Hawthorn (Crataegus Azarolus) of Iraqi Kurdistan Region by HPLC Analysis

Baram Ahmed Hamahameen and Banaz Jamal

***Abstract*—Flavonoids content of two types of hawthorn leaves (crataegus azarolus) which grows in Iraqi Kurdistan region (season 2011) including Zalan and Qaradax, were determined by RP-HPLC technique. The percentages of major flavonoids contents were found to be Hypersoide and Vitexin-rahmnose(28.12-30.26 %), (30.30-18.00%), respectively and these results were compared with ratio of them in the hawthorn fruits of the same plants in which these two majors (Hypersoide and Vitexin-rahmnose) flavonoids became very low but some other flavonoids like Querecetin, Nonacosan-10-01 and Apigenin show high percentage (18.24%, 13.72% and 11.98% respectively) in Hawraman hawthorn and in Qaradax hawthorn some other types are dominated like Nonacosan-10-01, Kaempferol and Querecetin having following ratio (18.10%, 16.59% and 15.51% respectively). Divert compounds of flavonoids composition were analyzed also by HPLC technique in these two types of hawthorn leaves, different ratio of : 1-Neoategolic acid, 2-Kaempferol (I), 3-Querecetin, 4-Nonacosan10-01 (II), 5-Apigenin(III), 6-Luteolin(IV), 7-Rutin(V), 8-Hypersoide(VI) and 9-Vitexin-rahmnose (VII), these compounds have main physiological role and pharmaceutical effect on the fruits of Hawthorn and also same role on the animal and human physiology.**

***Index Terms*—Crataegus *Azarolus*, flavonoids, HPLC analysis, Hawthorn.**

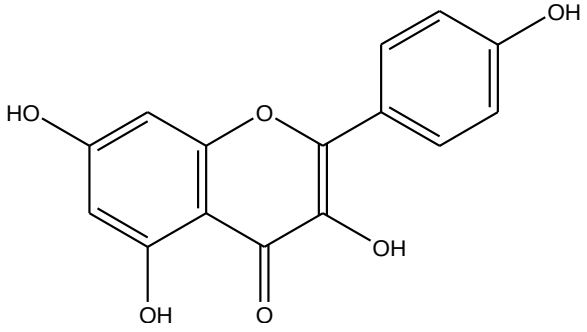
I. Introduction

Our previous work in this field indicates the percentage of fatty acids and triterpinoids contents of hawthorn fruits but in this new work we want to show the flavonoids ration in hawthrorn leaves of the same region, Fig. 1[1].

Previous literature studies[2] identified hyperoside (HP), isoquercitrin (IQ) and epicatechin (EC) to be the major active flavonoid components of the hawthorn phenolic extract from hawthorn fruits demonstrating inhibitory effect on in vitro Cu(+2)-mediated low density lipoproteins oxidation. Hawthorn leaves from two different part of Kurdistan region of Iraq (Zalan and Qaradagh) have been collected, in order to study and investigate the flavonoids content by means of HPLC methods, the contents of the flavonoids in hawthorn leaves extract that disposed through different conditions were measured and compared and analyzed by using the ultraviolet-visible spectrophotometry with two methods. Results: the concentration of the flavonoids was 86.15% by using the $Al(NO_3)_3$(510 nm),and $AlCl_3$(272 nm) was 35.19% [3]. Hawthorn extract for treating chronic heart failure has been used by meta-analysis of randomized trials by a group of researchers and the results shows there is a significant benefit from hawthorn extract as an adjunctive treatment for chronic heart failure [4].

Jakstas *et al.* [5] worked on hawthorn flavonoids contents of some Lithuanian hawthorn they founded: Akmene (V1, V2, V3), Kedainiai (S1, S2, S3), Klaipeda (M1), Lazdijai (D1, D2, D3) and Vilnius (VL1, VL2) components were extracted with ethanol and flavonoids were measured by spectroscopic method and by high performance liquid chromatography. The ratio 0.72 to 1.89% of flavonoids were founded in the hawthorn buds. The dominating flavonoid is vitexin-2-O-rhamnosid (6.72-10.91 milligrams in one gram of dried crude drug). Other flavonoids are: vitexin: 0.88-6.53 mg/g, hyperosid: 0.85-2.70 mg/g, rutin: 0.72-2.10 mg/g and quercitrin: 0.82-1.01 mg/g.

Fig. 1. Hawthorn leveas of Zalan,Kurdistan North of Iraq Kaempferol.



Kaempferol (I)

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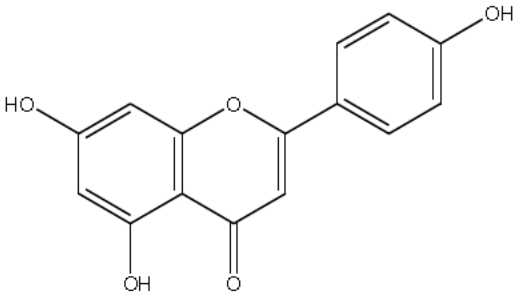
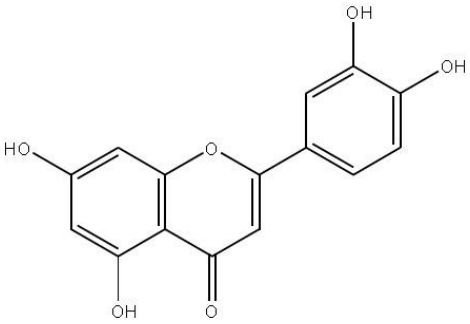


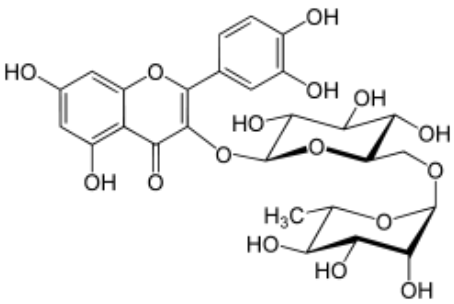
Apigenin (III)



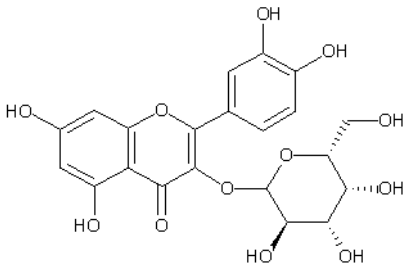
Luteolin (IV)

Nonacosane (II)

Rutin(V): Rutin, also called rutoside, quercetin-3-O-rutinoside and sophorin, is the glycoside between the flavonol quercetin and the disaccharide rutinose (α-L-Rhamnopyranosyl-(1→6))-β-D-glucopyranose). In fava d'anta, the synthesis is done via a rutin synthase activity[6], Rutin inhibits platelet aggregation[7], Luteolin IV shows anti-inflammatory activity in some animal and *in vitro* models[8], Rutin, as ferulic acid, can reduce the cytotoxicity of oxidized LDL cholesterol and lower the risk of heart disease and Rutin is also an antioxidant[9].



Rutin (V)

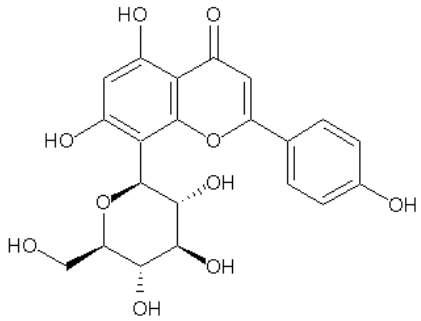


Hypersoide (VI)

II. Cancer Benefit

Vitexin is a flavonoid glycoside found in certain herbs including hawthorn herb.

Vitexins, nature-derived lignan compounds, induce apoptosis and suppress tumor growth. Lignans such as secoisolari- ciresinol diglucoside in flaxseed, are metabolizes to bioactive mammalian lignans of END and ENL. Because mammalian lignans have chemical structural similarity to the natural estrogen, they are thought to behave like selective estrogen receptor modulators and therefore have anticancer effect against hormone-related cancers. We isolated a series of lignan compounds, named as Vitexins, from the seed of Chinese herb Vitex Negundo. We purified several Vitexin lignan compounds. Cytotoxic and antitumor effects were analyzed in cancer cells and in tumor xenograft models. In vivo metabolism of Vitexins was determined in rat. Vitexin is a class of nature lignan compounds, whose action and anticancer effect is mediated by the mechanisms different from the classic lignans. Vitexin-induced antitumor effect and cytotoxic activity is exerted through proapoptotic process, which is mediated by a decreased Bcl-2/Bax ratio and activation of caspases.



Vitexin important (VII)

A. Material and Method

Two types of hawthorn [cartaegus azarolus] leaves, [Zalan and Qaradax] were harvested by hand in its optimum state for two consecutive seasons in 2011 in Kurdistan region –north Iraq, Zalan and Qaradax Fig. 1. After a morphological and chemical characterization, the samples were prepared for determination of their flavonoids constituents.

B. Preparation of the Sample for the Determination of Flavonoid Contents in Hawthorn Leaves and Fruits

The two types of hawthorn leaves Zalan and Qaradax were oven-dried at 50 ^{0}C for 18h and ground through a wiley mill to pass a 30-40 mesh screen, and stored tightly at 4 ^{0}C. 10g of the samples were dissolved in 30ml methanol ,then agitated in ultra sonic bath for 30minutes, then extract were filtered on whatman filter paper 0.5μm to remove the fibers and un-dissolved texture, the extract were pre-concentrated by stream of N2 to about 0.5ml and then complete the volume to 1ml by the mobile phase, then 20ul of the aqueous filtrate were injected to HPLC column[10], Table I.

TABLE I: FLAVONOIDS CONTENTS IN ZALAN HAWTHORN LEAVES

no	Flavor- noids	Rt/m	area/standard	df	area/sample	conc. µg/ml	conce. of sample	%
1	Neoategolic acid	0.98	30978	3	10232	25	24.7724	2.99447124
2	Kaempferol	1.83	34390	3	16662	25	36.3376	4.39246131
3	Querecetin	3.06	39894	3	12329	25	23.1783	2.80177502
4	Nonacosan-10-01	4.07	41009	3	15236	25	27.8646	3.36825354
5	Apigenin	4.89	62694	3	65552	25	78.4190	9.47922774
6	Luteolin	5.68	47283	3	45375	25	71.9735	8.70010721
7	Rutin	6.49	51741	3	56148	25	81.3881	9.83812788
8	Hypersoide	7.39	60515	3	187712	25	232.6431	**28.1217271**
9	Vitexin-rahmnose	8.4	51494	3	172124	25	250.6952	**30.3038489**
	Total						827.2719	

TABLE II: FLAVONOIDS CONTENTS IN QARADAX HAWTHORN LEAVES

no	Flavor- noids	Rt/m	area/standard	df	area/sample	conc. µg/ml	conce. of sample	%
1	Neoategolic acid	0.98	30978	3	13960	25	33.798	3.53336007
2	Kaempferol	1.83	34390	3	42689	25	93.099	9.73284170
3	Querecetin	3.06	39894	3	31704	25	59.602	6.23106569
4	Nonacosan-10-01	4.07	41009	3	41301	25	75.534	7.89654732
5	Apigenin	4.89	62694	3	62694	25	75.0000	7.840718364
6	Luteolin	5.68	47283	3	0	25	0.0000	0
7	Rutin	6.49	51741	3	108875	25	157.81	16.4986801
8	Hypersoide	7.39	60515	3	233590	25	289.50	**30.2654449**
9	Vitexin-rahmnose	8.4	51494	3	118224	25	172.19	**18.0013416**
	Total						956.545	

III. RESULT AND DISCUSSION

Hawthorn leaves flavonoids contents of [Zalan and Qaradax] were determined and it shown that the content of Hypersoide and Vitexin-rahmnose are (28.12-30.26 %), (30.30-18.00%), respectively. The characteristics properties of the flavonoids compared with their contents in their fruits which shown to be very different, there are some other flavonoids like Querecetin, Nonacosan-10-01 and Apigenin show high percentage (18.24%, 13.72% and 11.98% respectively) in Hawraman hawthorn and in Qaradax hawthorn some other types are dominated like Nonacosan-10-01, Kaempferol and Querecetin having following ratio (18.10%, 16.59% and 15.51% respectively) as in Tables I-IV, in addition there is a specific flavonoid Luteolin which have some mysterious results exist in the hawthorn leaves but disappear in its fruit (Zalan one) but in Qaradax simple this flavonoid is 0% in its leave but it appear in the hawthorn fruit of this Town.

The HPLC analysis of fatty acid composition in the two types of hawthorn leaves are presented in Table I and II from the result the each types of hawthorn leaves contain high percentage of Hypersoide and Vitexin-rahmnose, which is about 28.12% and 30.30% respectively in a Zalan hawthorn leaves and about 30.26% and 18% respectively in hawthorn leaves of Qaradax.

IV. CALCULATION

Concentration of sample µg/ml =

Area of sample /area of standard × Con.of standard × dilution factor

TABLE III: FLAVONOIDS CONTENTS IN QARADAX HAWTHORN FRUITS[1]

no	Flavor- noids	Rt/m	area/standard	df	area/sample	conc. µg/ml	conce. of sample	%
1	Neoategolic acid	1.98	25007	3	30716	25	92.1222	9.59131302
2	Kaempferol	2.66	41927	3	63380	25	113.3756	11.8041154
3	Querecetin	3.38	50898	3	78125	25	115.1199	**11.9857251**
4	Nonacosan-10-01	4.07	41431	3	72802	25	131.7890	**13.7212252**
5	Apigenin	5.14	21142	3	49406	25	175.2649	**18.2477207**
6	Luteolin	6.23	23729	3	20738	25	65.5464	6.82436794
7	Rutin	6.99	44471	3	50339	25	84.8963	8.83899094
8	Hypersoide	7.98	21863	3	27170	25	93.2054	9.70409152
9	Vitexin-rahmnose	8.75	31853	3	37865	25	89.1557	9.28245002
	Total						960.4754	

TABLE IV: FLAVONOIDS CONTENTS IN HAWRAMAN HAWTHORN FRUITS[1]

no	Flavor- noids	Rt/m	area/standard	df	area/sample	conc. µg/ml	conce. of sample	%
1	Neoategolic acid	1.98	25007	3	28535	25	85.5810	10.5032432
2	Kaempferol	2.66	41927	3	75569	25	135.1796	**16.5904064**
3	Querecetin	3.38	50898	3	85808	25	126.4411	**15.5179447**
4	Nonacosan-10-01	4.07	41431	3	81477	25	147.4928	**18.1015912**
5	Apigenin	5.14	21142	3	24859	25	88.1858	10.8229268
6	Luteolin	6.23	23729	3	0	25	0.0000	0
7	Rutin	6.99	44471	3	14524	25	24.4946	3.00619040
8	Hypersoide	7.98	21863	3	29265	25	100.3922	12.3209987
9	Vitexin-rahmnose	8.75	31853	3	45460	25	107.0386	13.1366983
	Total						814.8058	

The separation occurred on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump model LC-10AV Shimadzu, the eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer.

V. CONCLUSION

The two types of hawthorn leaves [Zalan and Qaradax] in Iraqi Kurdistan region are rich source of Hypersoide and Vitexin- rahmnose (according to HPLC analysis) which both are the main effectives flavonoids in Hawthorn leaves as mentioned in the introduction for medical purpose for example Vitexin (VII) is a class of nature lignan compounds, whose action and anticancer effect is mediated by the mechanisms different from the classic lignans. Vitexin-induced antitumor effect.

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