

Identification of Artemisinin compound in *Artemisia herba alba* belong to the Asteracea by HPLC and GC/MS

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Summary

This study emphasized on the detection and identification of Artemisinin flavonoid in *Artemisia herba alba*, the medicinal importment acquired this study it is importment Artemisinin was identified in plant extract of aerial parts by chromatographic methods Gas chromatography and mass spectro (GC/MS) this technique the identified active compounds and High performance liquid chromatogram (HPLC) this the technique the proved the presence of Artemisinin concentratin in flavonoid extract by identical retention time .

Key words: *Artemisia herba alba* , Artemisinin , Flavonoid , HPLC, GC/MS.

Introduction:

Artemisia herba alba plant (Asteracea family commonly known as annual wormwood) is a plant used many centries in medicine treatment for many of diseases (1) , Moreover , *Artemisia herba alba* leave a high content of cineole , Artemisia ketone , camphene (2) , *Artemisia* have been used in Traditional medicine, These species have been used as antimicrobial, antispasmodic, and blood coagulation (3). The population of some countries of the Middle East is also used as an anti-diabetic drug (4), Some species also showed anti-malarial effect and antimicrobial 5, 6) .That represent a rich source of variouse phenolic compounde as asource of natural antioxidant (7). Such flavonoid have great potention to inhibit the generation of reactive oxygen speciese (ROS) and once they are formed to perform antioxidant function , flavonoid are found in the chloroplast which play a role as scavenger of oxygen and stabilizers of chloroplast outer envelope memberan (8).

Moreover , The main chemical constituents of *Artemisia* are sesquiterpenoids include artemisic acid , artemisinol , artemisilactone , epoxyarteannuiunic acid , artemisinin I , artemisinin II, artemisinin III, artemisininIV, artemisininV (9) . Artemisinin (AN) is a secondary metabolite of *Artemisia* and Artemisinin was given the name Qinghaosu, which means “principle from Qinghao”, with an endoperoxide bridge as depicted in Fig(1), which is has activity against malaria (10, 11). Artemisinin found in glandular trichomes of floral buds and flowers (12, 13) ,

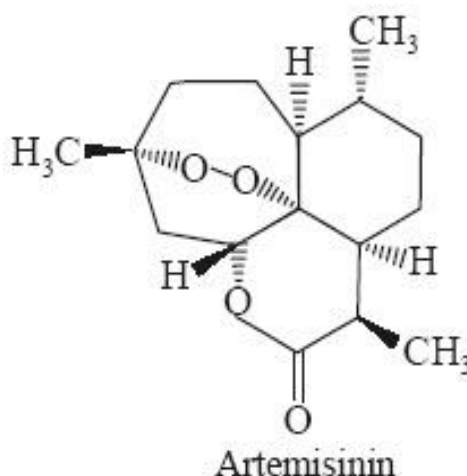


Fig. (1) Chemical structure of artemisinin.

Materials and methods

1:plant collection

A. herba alba obtained from Karbalaa city in 2018 during the flowering stage. The plant taxonomy .the plant were air dried at oven degree 45C° and powdered.

2:Soxhlet Extraction

50 gm of fin powder was plased in thimble and extracted with 50 ml of (70%) methanol in flask round volum (500 ml) for 24 hours, (14) obtained extract was then evaporated at (45 C°) using a rotary, The extract dried by freez drayer (lyophilizer), dried extract then collected , weighted and kept in freeze at (-20C°) Until use.

4: Extrraction of total flavonoids from *A. herba alba*.

Twenty gram from dried methanol extract was placed in reflex apparatus for 8 hr using 400 ml of 2M HCL solution , the filtrate was cooled and transferred to a separator funnel , the aglycon moiety was extracted by 100 ml ethy acetate . the collected ethyl acetate layers were washed with distilled water to eliminate the excess acid then evaporated to dryness by rotary evaporator at 40C° in 20 mint. (15).

5: Identification of Artimisinin compound by HPLC :

HPLC analysis of flavonoid in ministry of science and Technology / Baghdade . with column (C18-RP 25cm ×4.6 mm ID (5.0_m pore size), kept at room temperature, The mobile phase was isocratic and constituted of water, adjusted to pH 3.0–3.5 with Trifluoro acetic acid (TFA) :acetonitrile (65:35) at the flow rate of 1.0 ml/min with detection limited 250 nm. (16).

Calculation :

Concentration of sample = Area of sample / Area of standard X con. of standard X dilution factor .

Con. of sample = 13261.644/678.554 * 25*10= 4885ppm.

Results:

1- Gas chromatochraphy and mass spectro:

Gc/mass analysis of compounds in extraction of *A. herb alba* showed 78 peaks of compound detected, The major compound present were : 9-octadecenoic

acid , methyl ester , 8- octadecenoic acid , methyl ester , (E) and gama.- Sitosterol (99%) in *A. herba alba* . in figure (2) , table (1) .

2- Identification of Artemisinin by High performance liquid chromatogram (HPLC)

HPLC analysis Artemisinin standerd and Flavonoid of air parts of *Artemisia herba alba* successfully provide present compound with retention time was 3.45 min figure (2) and table (2) wich have Artemisinin is 25 ppm .

Artemisinin content in the Flavonoid 488 ppm as show in figure (3) and table (3).

The following charts were obtained .

Discusse

Previous studies analysis of *A. herba alba* fruit by GC-MS method recorded the presence of 21 compound use hexan and ethyl acetat (1:1) Where it found high ratios of turbines, essential oils, alkaloids and phenolic (17), (18) a total of 45 constituents in essential oil of *Artemisia indiica* constituting 97.6% of the total oil composition. The oil composition is dominated by the presence of oxygenated monoterpenes constituting 65.2% of the total oil composition followed by sesquiterpene hydrocarbons (16.5%), and monoterpene hydrocarbons (10.7%). The principal chemical constituents were found to be artemisia ketone (42.1%), germacrene D (8.6%), borneol (6.1%), chrysanthenyl acetate (4.8%), p-cymene (2.7%), a-thujone (2.7%) and b-pinene (2.4%). The essential oil composition of *A. vestita* was found to contain a total of 18 components representing 94.2% of the total oil composition. The principal components were found to be 1,8-cineole, (E)-citral, limonene, a-phellandrene, camphor, (Z) and (E)-thujones. Oxygenated monoterpenes were the dominant group of terpenes in the essential oil constituting 73.1% of the total oil composition followed by monoterpene hydrocarbons (17.3%).

Previous studies such as (19) indicate the Artemisinin in the herb of *Artemesia annua* L was 0.625%.

(16) found Artemisinin in the plant extrct of presence *A. annua* 0.3mg\ml at peak 6.07min, Artemisinin levels found in the flowering tops and leaves but not in root because it is affected by some factors such as growth condition , seasonal and geographical variation such as breeding (20).

(21) reported extremely high artemisinin concentration (4.85–4.90% w/w) in their study performed in the Egyptian desert for the first time as a new promising cultivating area for pharmaceutical production of artemisinin .

Figur (2) Identified components of methanolic extract of *A. herba alba* using GC-MS.

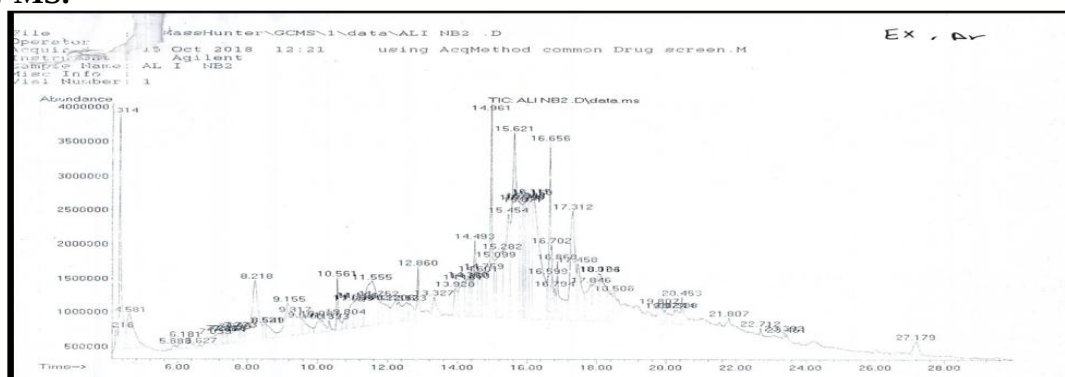
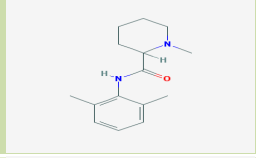
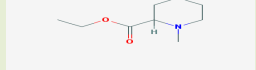
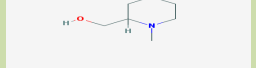
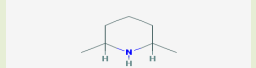
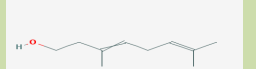
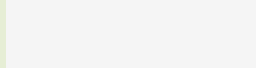
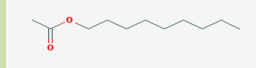
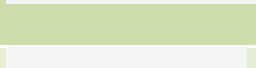
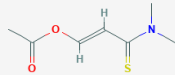
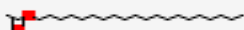
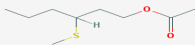
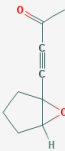
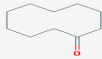
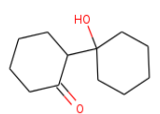
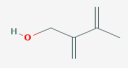
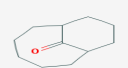
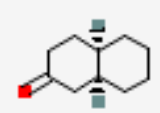
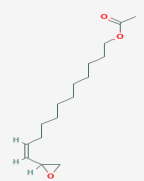
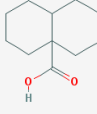
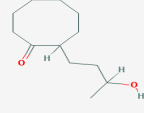
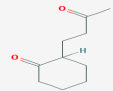
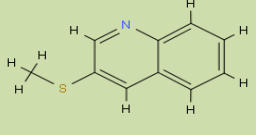
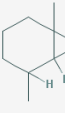


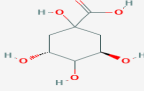
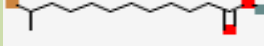
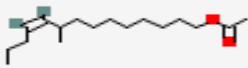
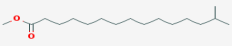
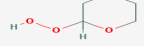
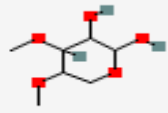
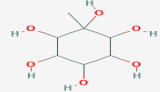
Table (1): Identified components of methanolic extract of *Artemisia herba alba* using GC-MS.

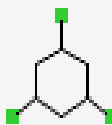
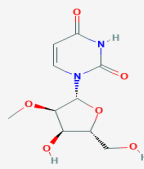
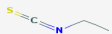
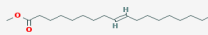
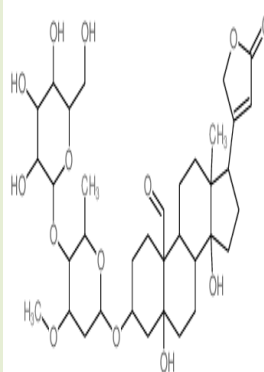
Peak	R.T	Ar%	Chemical Names	Molecular Formula	Molecular Weight	Structure
1	4.215	0.39	Mepivacaine	C ₁₅ H ₂₂ N ₂ O	246.354 g/mol	
2	4.313	3.66	Ethyl 1-methylpipercolinate	C ₉ H ₁₇ O ₂	171.24 g/mol	
3	4.584	2.12	1-Methyl-2-piperidinemethanol	C ₇ H ₁₅ NO	129.203 g/mol	
4	5.880	0.11	Nanofin	C ₇ H ₁₅ N	113.204 g/mol	
5	6.182	0.76	3,6-Octadien-1-ol, 3,7-dimethyl-, (z)-	C ₁₀ H ₁₈ O	154.253 g/mol	
6	6.626	0.02	Tridecyl acetate	C ₁₅ H ₃₀ O ₂	242.403 g/mol	
7	7.041	0.49	Acetic acid, nonyl ester	C ₁₁ H ₂₂ O ₂	186.295 g/mol	
8	7.229	0.47	n-PROPYL DECYL ETHER	C ₆ H ₁₄ O	102.177 g/mol	
9	7.274	0.12	Acetic acid, decyl ester	C ₁₂ H ₂₄ O ₂	200.322 g/mol	

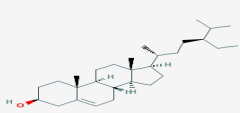
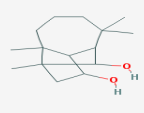
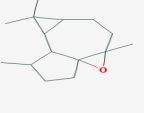
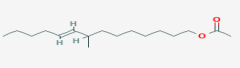
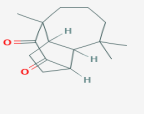
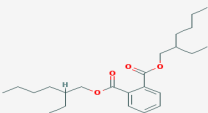
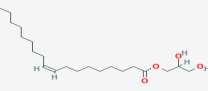
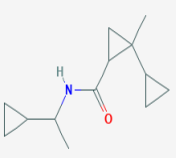
11	7.433	0.13	2-Decenal , (E)-	$C_{10}H_{18}O$	154.253 g/mol	
12	7.478	0.24	2-propenethioamide, 3-(acetyloxy)-N,N-dimethyl-,(E)-	$C_7H_{11}NO_2S$	173.23 g/mol	
13	7.598	0.19	Eicosyl acetate	$C_{22}H_{44}O_2$	340.592 g/mol	
14	7.734	0.38	3-(Methylthio) hexyl acetate	$C_9H_{18}O_2S$	190.301 g/mol	
15	7.772	0.14	Pentanedioic acid, monomethyl ester	$C_6H_{10}O_4$	146.142 g/mol	
16	8.216	3.44	Phenol, 3-ethyl-	$C_8H_{10}O$	122.167 g/mol	
17	8.518	0.30	1-Hexadecanol, acetate	$C_{18}H_{36}O_2$	284.484 g/mol	
18	8.548	0.62	4-(6-oxabicyclo[3.1.0] hex-1-yl) but-3-yn-2-one	$C_9H_{10}O_2$	150.177 g/mol	
19	9.158	1.77	Lilac alcohol A	$C_{10}H_{18}O_2$	170.252 g/mol	
20	9.316	1.20	Cyclodecanone	$C_{10}H_{18}O$	154.253 g/mol	

21	9.580	0.51	1Hydroxy-p-menth-3-one	$C_{10}H_{18}O_2$	170.25g/mol	
22	10.078	0.49	3-Buten-1-ol, 3-methyl-2-methylene	$C_6H_{10}O$	98.145 g/mol	
24	10.334	0.17	Trichloroacetic acid , Tetradecyle ester	$C_{16}H_{29}Cl_3O_2$	359.756 g/mol	
25	10.560	0.84	Undecanoic acid , 10-methyl-, methyl ester	$C_{13}H_{26}O_2$	214.349 g/mol	
26	10.801	0.28	2- Heptanone , 6-methyl-	$C_8H_{16}O$	128.215 g/mol	
27	11.012	0.99	Bicyclo[5.3.1]undecan-11-one ,	$C_{11}H_{18}O$	166.264 g/mol	
29	11.117	0.56	Cyclohexanone, 2-ethyl-	$C_8H_{14}O$	126.199 g/mol	
30	11.155	0.74	2(1H)-Naphthalenone, octahydro-4a-methyl-, trans-	$C_{10}H_{16}O$	152.237 g/mol	
31	11.283	0.34	z-(13, 14-Epoxy) tetradec-11-1-ol acetate	$C_{16}H_{28}O_3$	268.397 g/mol	

32	11.554	3.75	4a(2H) – Naphthalenecarboxylic acid, octahydro-cis	$C_{11}H_{18}O_2$	182.263 g/mol	
33	11.750	0.57	2-cyclohexan-1-ol, 1-methyl-4-(1-methylethylen)-, trans-	$C_{10}H_{18}O$	154.253 g/mol	
34	12.210	0.85	Bicyclo[2.2.2] oct-2-ene, 1,2,3,6-tetramethyl-	$C_{12}H_{20}$	164.292 g/mol	
35	12.315	0.62	2-(3-Hydroxybutyl) cyclooctanone	$C_{12}H_{22}O_2$	198.306 g/mol	
36	12.534	0.71	Cyclohexanone, 2-(3-oxobutyl)-	$C_{10}H_{16}O_2$	168.236 g/mol	
37	12.858	0.79	Methyl tetradecanoate	$C_{15}H_{30}O_2$	242.403 g/mol	
39	13.928	0.76	Quinoline, 3-(methylthio)-	$C_{10}H_9S$	175.04 g/mol	
40	14.162	1.45	7-Oxabicyclo[4.1.0]heptane, 1,5-dimethyl-	$C_8H_{14}O$	126.199 g/mol	
41	14.297	1.17	Trichloroacetic acid, hexadecyl ester	$C_{18}H_{33}Cl_3O_2$	387.81 g/mol	

42	14.350	0.61	Quinic acid	$C_7H_{12}O_6$	192.167 g/mol	
44	14.599	1.37	11-Bromoudecanoic acid	$C_{12}H_{23}BrO_2$	279.218 g/mol	
45	14.757	1.42	9-Methyl- α -10-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.441 g/mol	
46	14.960	4.11	Pentdecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.457 g/mol	
47	15.096	0.95	Decanoic acid, 3-methyl -	$C_{11}H_{22}O_2$	186.295 g/mol	
48	15.284	2.82	Hydroperoxide, 1,4-dioxan-2-yl	$C_4H_8O_4$	120.104 g/mol	
50	15.623	8.07	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43 g/mol	
51	15.774	2.58	1,3-Dioxolane, 2-methyl-2-propyl-	$C_7H_{14}O_2$	130.187 g/mol	
52	15.872	0.71	3,4-Di-O-methyl-L-arabinopyranose	$C_7H_{14}O_5$	178.184 g/mol	
53	15.910	1.06	Myo-Inositol, 2-C-methyl-	$C_7H_{14}O_6$	194.183 g/mol	

55	15.963	2.36	Cyclohexane, 3R-acetamido-4 cis, 6cis-bis(acetoxy)- 5 trans-dimethylamino-	$C_6H_9Cl_3$	187.488 g/mol	
57	16.151	7.55	Uridine, 2'-o-methyl-	$C_{10}H_{14}N_2O_6$	258.23 g/mol	
58	16.603	0.43	Ethane , isothiocyanato	C_3H_5NS	87.14 g/mol	
59	16.656	2.00	9- octadecenoic acid, methyl ester , (E) -	$C_{19}H_{36}O_2$	296.495 g/mol	
61	16.791	0.45	Card-20(22)- enolide, 3-[(2,6-dideoxy-4-o- .D-glucopyranosyl-3-o-methyl-.beta.- D-ribo-hexapyranosyl) oxy]-5,14-dihydroxy-19-oxo-,3.beta.,5.beta.]	$C_{36}H_{54}O_{14}$	710.80600	
62	16.867	1.20	Methyl stearate	$C_{19}H_{38}O_2$	298.511 g/mol	
63	17.311	4.37	Trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.468 g/mol	

64	17.455	2.28	Cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.468 g/mol	
65	17.846	1.64	.gamma.- Sitosterol	$C_{29}H_{50}O$	414.718 g/mol	
68	18.509	0.90	Culmorin	$C_{15}H_{26}O_2$	238.371 g/mol	
69	19.806	0.83	Ledone oxide-(II)	$C_{15}H_{24}O$	220.356 g/mol	
70	19.979	0.19	E-8-Methyl-9-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268.441 g/mol	
72	20.326	0.45	1,2-Longidione	$C_{15}H_{22}O_2$	234.339 g/mol	
73	20.454	0.74	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.564 g/mol	
74	21.810	0.16	9-Octadecenoic acid (z) -, 2,3-dihydroxypropyl ester	$C_{21}H_{40}O_4$	356.547 g/mol	
75	22.714	0.08	Cyclopropane carboxamide, 2-cyclopropyl-2-methyl-N-(1-cyclopropylethyl)-	$C_{13}H_{21}NO$	207.317 g/mol	

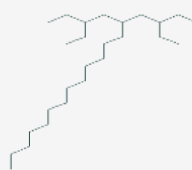
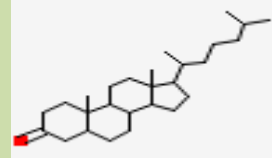
76	23.392	0.09	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366.718 g/mol	
77	23.460	0.11	Cholestan-3-one, 4,4-dimethyl -, .alpha .)-	$C_{27}H_{46}O$	386.664 g/mol	
78	27.183	0.60	Oxirane, decyl-	$C_{12}H_{24}O$	184.323 g/mol	

Fig (3) HPLC of standard Artemisinin

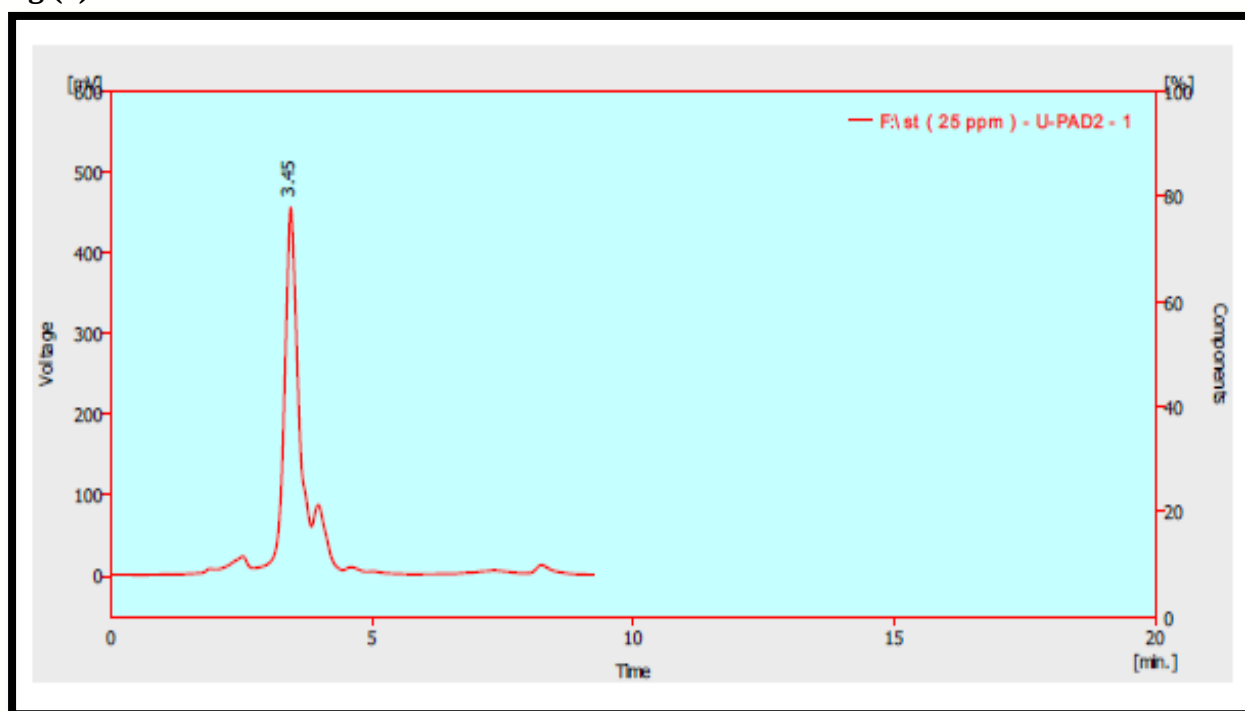


Table (2) HPLC of sandared Artemisinin

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Chromatogram F:\st (25 ppm).PRM

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Result Table (Uncal - F:\st (25 ppm) - U-PAD2 - 1)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.448	678.554	112.036	100.0	100.0	0.10	
	Total	678.554	112.036	100.0	100.0		

Fig (4) HPLC of artemisinin in flavonoid .

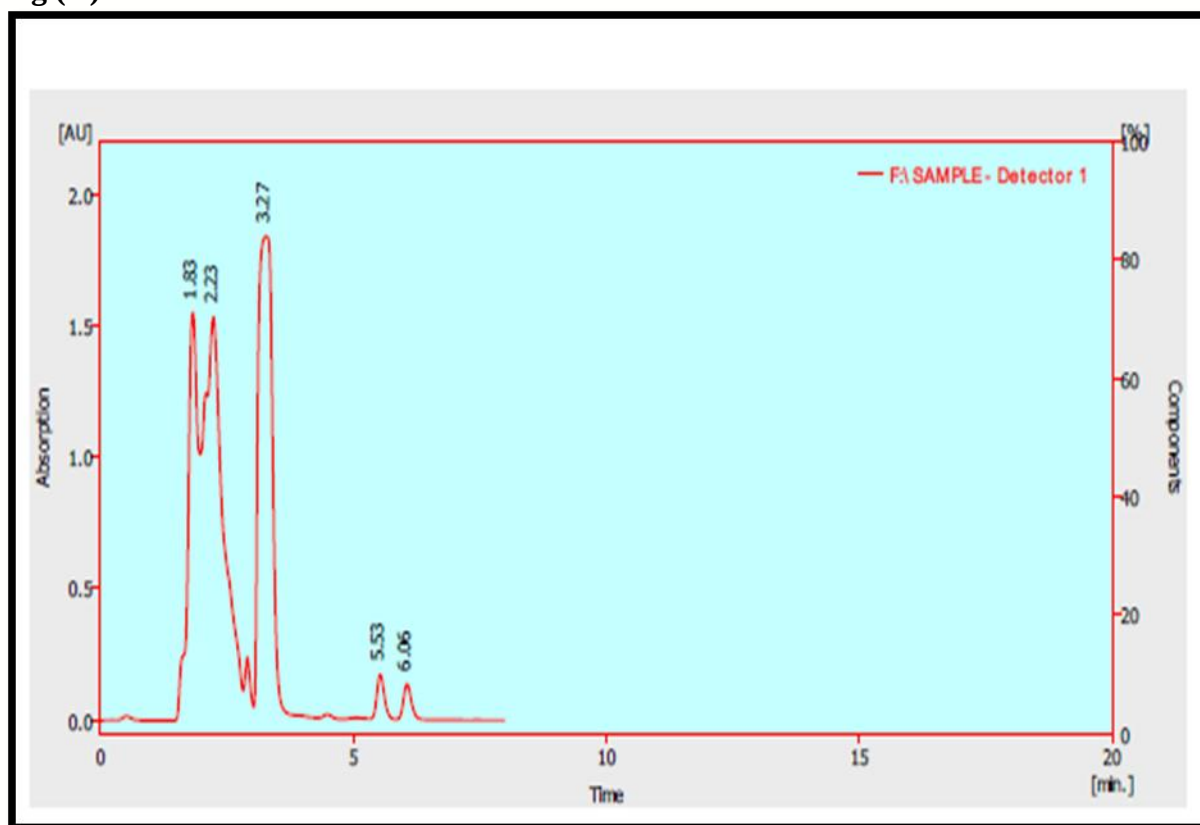


Table (3) HPLC of artemisinin in flavonoid .

03/10/2018 09:03 م

Chromatogram F:\SAMPLE.PRM

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Result Table (Uncal - F:\SAMPLE - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Peak Purity [-]	Compound Name
1	1.825	5366.367	688.554	22.2	31.6	0.14	955	
2	2.233	4060.121	415.583	16.8	19.1	0.15	985	
3	3.267	13261.644	889.701	54.9	40.8	0.27	963	
4	5.525	537.505	75.829	2.2	3.5	0.10	994	
5	6.058	924.202	109.164	3.8	5.0	0.15	974	
	Total	24149.838	2178.831	100.0	100.0			

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