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CHROMATOGRAPHIC INVESTIGATION OF ALKALOIDS (ATROPINE AND HYOSCINE) AND FATTY ACID COMPOUNDS OF *DATURA STRAMONIUM* AND STUDY OF ITS ANTIOXIDANT EFFECT

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Abstract

The importance of antioxidants in reducing the risk of free radicals and the reduction of diseases in humans, the current study was interested in obtaining natural antioxidants through the separation of alkaloids (atropine and hyoscine) and fatty acids of *Datura stramonium*. And then study the antioxidant efficacy of these compounds and compare them with ascorbic acid by using the DPPPH method. Investigation by HPLC the results show that the datura contains the alkaloids (hyoscine and atropine), while, The identification of the petroleum ether extract by GLC showed the presence of the fatty acids: (Caproic acid, Undecanoic acid, Pentadecanoic acid, Archidic acid, Heneicosanoic acid and Behenic acid). And The results showed that alkaloids had the highest free-radical (DPPH) inhibitor concentration at (500µg/ml) in the rate of (40.8%) while fatty acids had the highest inhibition ratio (32.22%) at the same concentration, While the highest inhibition of ascorbic acid (82.6%) at the concentration of (500µg/ml) and the lowest inhibitory rate of free radicals (62.54%) at the concentration of (200µg/ml). The results show the importance of alkaloids and fatty acids in the free radical inhibition, and the rate of free radical inhibition increases with the high concentration of antioxidant compounds.

Introduction

Medicinal plants have been used for centuries as a treatment for human diseases because they contain chemical components of therapeutic value. According to the World Health Organization (WHO), about 65-80% of the world's population in developing countries depends mainly on traditional medicines as primary means of health care, 20-35% are mainly residents of developed countries also benefit indirectly from natural products in health care (Ghourchian *et al.*, 2016).

Antioxidants play an important role in preventing unwanted changes in food flavor, aroma and natural properties. It also reduces the risk of chronic diseases such as cancer, diabetes and inflammation (Vasundhara *et al.*, 2008). Therefore, based on WHO guidance and on the global trend towards the use of natural substances and their extracts, research has been directed towards isolating new types of natural and high-efficiency antioxidant (Barlow, 1990). Plant phenolic antioxidants have multiple properties, which act as an effective inhibitor of free radicals on the one hand, and enhance the body's immunity to diseases such as atherosclerosis (Yang, 2007). One of the important plants used in the medical field is *Datura stramonium*.

Datura stramonium is a triglyceride plant (C3) and it is an annual plant which belongs to the Solanaceae family (Salehian, 2012). The species of *Datura* can be found all over the world. It growths in sandy flats, plains and areas up to 2500 feet high above sea level (Singh *et al.*, 2003).

It is growing to a height of between 10-40cm. The flowers are large somewhat. The fruit is a large and very thorny and contains numerous black to dark brown seeds. The stems are slick, solid and mostly upright. The leaves are large, nearly 20cm long and oval like with a adverse and coarsely dentate margin. The root is long, thick and acuminating and somewhat branched (Oseni *et al.*, 2011).

Datura stramonium is a very important medicinal

plant, and it contains tropane alkaloids, amino acids, tannin, phytic acids, carbohydrates have been isolated (Singh, 2013). Its diverse biological activities include anti-asthmatic, antibacterial, antifungal (Couladis *et al., 2003*), larvacidal, antispasmodic, antioxidant, antinociceptive, anti-rheumatoid and anti-ulcer activities (Usha K *et al., 2009*).

The total concentration of alkaloids in *Datura stramonium* leaves between 0.2 – 0.5%. Morethan 70 alkaloids were specified in different parts of the plant, but the main alkaloids are: hyoscyamine, atropine and scopolamine (Fig. 1) (Reddy, 2009).

The aim of the study is to find natural alternatives as antioxidants by separating some alkaloids and fatty acids and studying their antioxidant effectiveness.

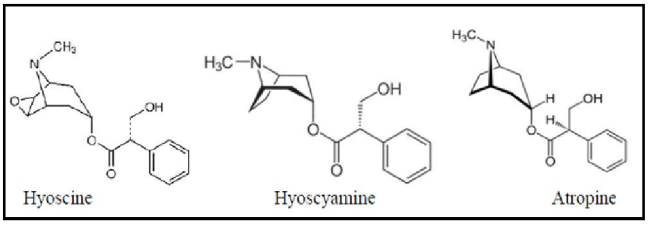


Fig. 1: Chemical structures of some tropane alkaloids.

Classification of *Datura stramonium*

Kingdom: Plants.

Subkingdom: Tracheobionta – Vascular plants.

Super division: Spermatophyta – Seed plants.

Division: Magnoliophyta – Flowering plants.

Class: Magnoliopsida – Dicotyledons.

Subclass: Asteridae.

Order: Solanales.

Family: Solanaceae – Potato family.

Genus: *Datura* L.

Species: *Datura stramonium* L.

(Gaire & Lalita 2013).

Materials and Methods

Plant leaves collection

The leaves of the Datura plant were collected from the Mosul dam area and were classified at the Medical Plants Development Center at Mosul Dam of the Iraqi Ministry of Agriculture. And then cleaned the leaves from the dust and so on, and dried on the papers away from the sun, taking into account flipping daily and monitored from rot or infected with pathogens such as fungi, and then placed in paper bags and kept in conditions away from moisture until use.

Preparation of some plant extracts using continuous soxhlet apparatus.

The leaves of the plant were crushed by an electric mill, and 25gm of well-ground powder was placed in the Batch in Soxhlet system, 400mL ether petroleum was added to extract the oil from the datura leaves. The extraction continued at a rate of 7 hours per day until the solvent used in the device became colorless then added Methylated spirit industrial (IMS) solvent (Ethanol 95% : Methanol 5%). Finally, concentrate the extract by rotary vacuum evaporator (Al-Daody, 1998).

Preparation of alkaloidal rich extracts

The IMS extract was triturated with 5% v/v of 0.5N HCl. The aqueous fraction was basified with ammonia solution up to pH 9. Then the solution was portioned with chloroform. The process was repeated for 5-6 time till the chloroform layer became colorless. The remaining aqueous layer was further fractioned with ethyl acetate. The same method was followed with chloroform. In the end there are three separate layers: chloroform, ethyl acetate and aqueous. Then, concentrate the extract by Rotary vacuum evaporator and the alkaloids were then detected by Dragendoff regent (Biswasroy *et al.*, 2017).

Saponification

Take 5 ml of the crude extract of the petroleum ether and added 100mL of 1N (KOH), Heating the solution for 90 minutes at 100°C, Then, added 100ml of distilled water and 50ml ether solvent and put in the separating funnel, and took the aqueous layer and added the concentrated sulfuric acid $H-_2SO_4$ until PH=2. In the end add 50ml of ether and put again in the separating funnel and take the organic layer and retain well (Arthur,1972).

Identification of Atropine and Hyoscine by HPLC technique

The Identification of alkaloids (Atropine and Hyoscine) was performed in the laboratories of the Ministry of Science and Technology/Department of Environment and Water, by HPLC model (SYKAM) Germany. Pump model: S 2100 quaternary gradient pump, auto sampler model: 5200, detector: UV (S 2340) and column oven model: (S4225). The mobile phase was:

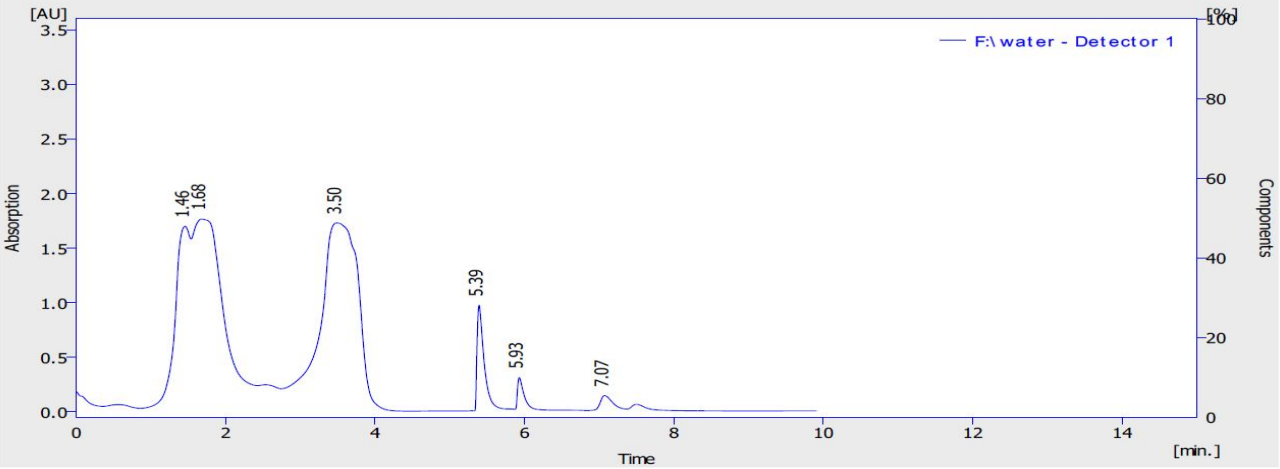
A= (Methanol: D.W.: acetic acid) (85:13:2).

B= (Methanol: D.W.: acetic acid) (25:70:5).

The column is C18-ODS (25 cm*4.6mm) and detector UV-369 nm at flow rate 1ml/min.

Identification of fatty acids by GLC technique

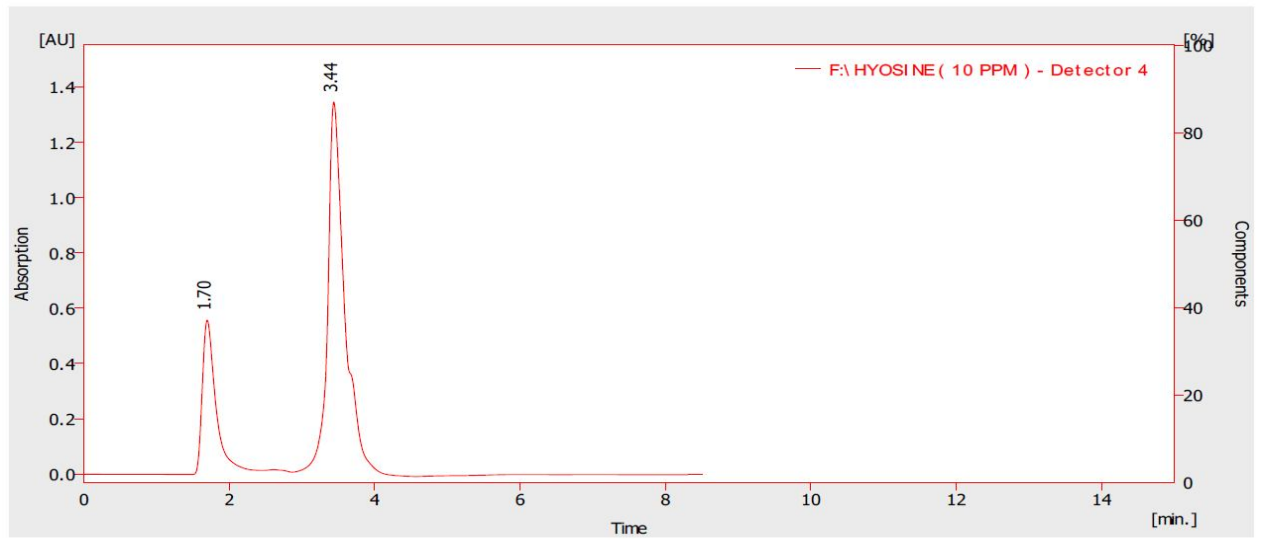
The separated fatty acids were diagnosed in the laboratories of the Ministry of Science and Technology /



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

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	1.460	192.899	52.692	2.6		
2	1.677	465.128	41.261	6.2		
3	3.497	4414.095	239.138	59.3		
4	5.393	1480.559	400.509	19.9		
5	5.930	682.756	162.290	9.2		
6	7.073	211.119	29.357	2.8		
	Total	7446.555	925.247	100.0		

Fig. 2: The curve for the sample of alkaloids atropine and hyoscine in the aqueous extract by HPLC.



Result Table (Uncal - F:\HYOSINE (10 PPM) - Detector 4)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	1.697	1559.955	229.319	40.1		
2	3.437	2334.035	299.543	59.9		
	Total	3893.990	528.863	100.0		

Fig. 3: Standard curve of Hyoscine compound by HPLC.

Department of Environment and Water by GLC model (Shimanezo) Japanese (2010) using ionized flame detector and Using the poetic column type (SE-30) wavelengths (0.25mm 0.5um, 30m) The temperature was in the injection area and the detector (330 and 280) While the column temperature gradually starts from (120-280) m At a rate of 8°/min using passive nitrogen gas as a carrier gas at a rate of 100 KP.

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 2, 2-diphenyl-1-picrylhyorazyl (DPPH). 1ml of 0.1m M DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (200, 300, 400 and 500µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100µg/ml) was used as reference standard. Mixer of 1mlmethanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer (Sumathy *et al.*, 2013). The ability to Inhibition the DPPH radical was calculated using the following formula:

$$\text{Radical scavenging effect}(\%) = (AbS - AbB)/AbB \times 100$$

AbS – Absorbance of Test Sample

AbB – Absorbance of blank

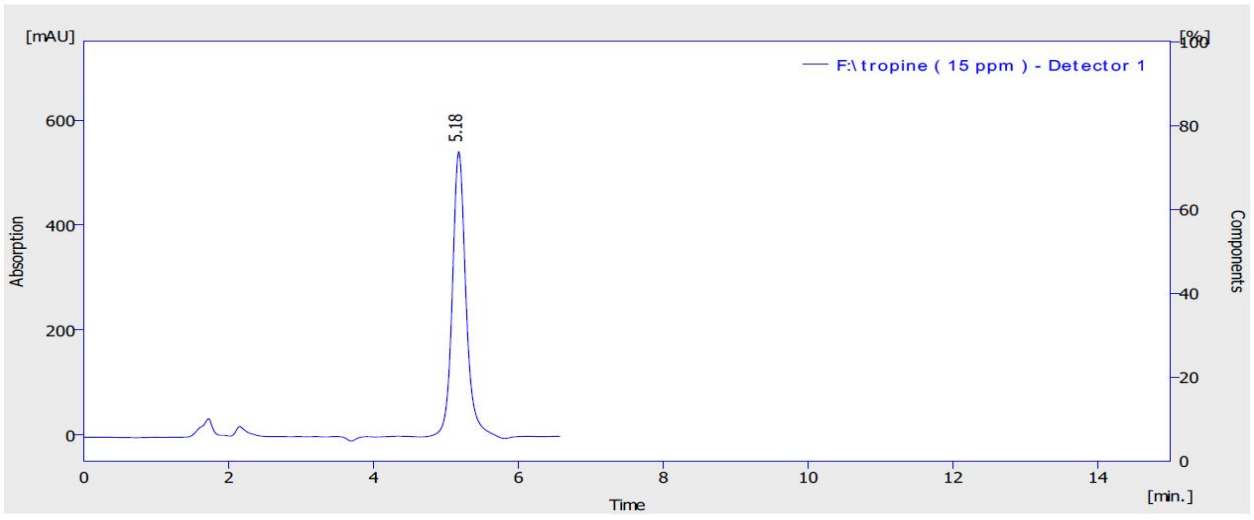
Results and discussion

Identification of alkaloid compounds; Atropine and Hyoscine by HPLC technique.

The investigation by hplc was shown the alkaline aqueous extract contained a Hyoscine compound at a time of retention (3.50) minutes Fig. 2, it is identical to the standard sample retention time (3.44) Fig. 3. While the Atropine compound appeared at the time of retention (5.39) minutes Fig 2, which corresponds to the standard detention time (5.18) minutes Fig. 4.

The identification of fatty acid compounds of *Datura stramonium* by GLC technique.

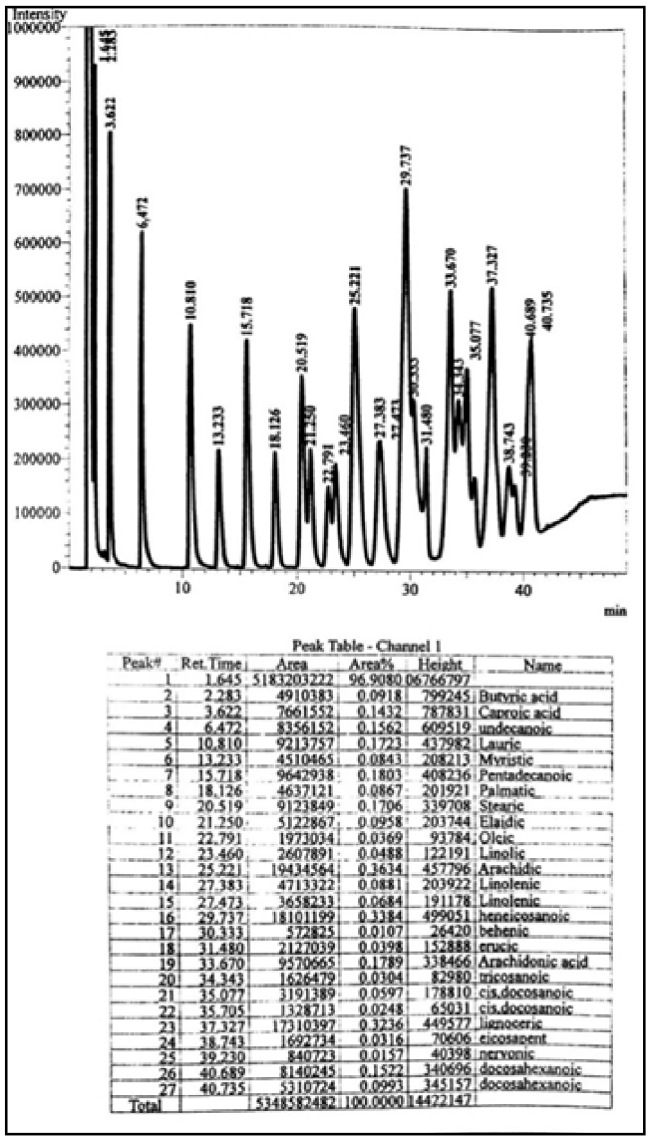
The Identification of the petroleum ether extract by GLC showed the presence of the following fatty acids Fig. 5, 6 : Caproic acid at a time of retention (3.622) minutes and corresponds to the standard compound at a time of retention (3.568) minutes. Undecanoic acid at a time of retention (6.418) minutes and corresponds to the standard compound at a time of retention (6.472) minutes. Pentadecanoic at a time of retention (15.550) minutes and corresponds to the standard compound at a time of retention (15.718) minutes. Archidic acid at a time of retention (25.085) minutes Fig. 6 and corresponds to the



Result Table (Uncal - F:\tropine (15 ppm) - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Compound Name	Correction Factor
1	5.177	507.004	118.849	100.0		
	Total	507.004	118.849	100.0		

Fig. 4: Standard curve of Atropine compound by HPLC.

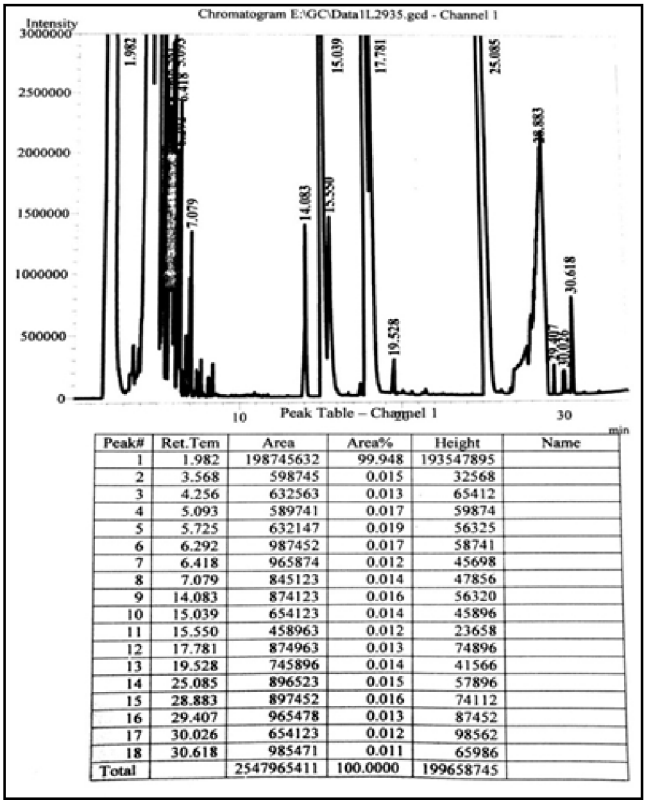


Peak Table - Channel 1

Peak#	Ret.Time	Area	Area%	Height	Name
1	1.645	5183203222	96.9080	06766797	
2	2.283	4910383	0.0918	799245	Butyric acid
3	3.622	7661552	0.1432	787831	Caproic acid
4	6.472	8356152	0.1562	609519	undecanoic
5	10.810	9213757	0.1723	437982	Lauric
6	13.233	4510465	0.0843	208213	Myristic
7	15.718	9642938	0.1803	408236	Pentadecanoic
8	18.126	4637121	0.0867	201921	Palmatic
9	20.519	9123849	0.1706	339708	Stearic
10	21.250	5122867	0.0958	203744	Elaidic
11	22.791	1973034	0.0369	93784	Oleic
12	23.460	2607891	0.0488	122191	Linolic
13	25.221	19434564	0.3634	457796	Arachidic
14	27.383	4713322	0.0881	203922	Linolenic
15	27.473	3658233	0.0684	191178	Linolenic
16	29.737	18101199	0.3384	499051	heneicosanoic
17	30.333	572825	0.0107	26420	behenic
18	31.480	2127039	0.0398	152888	erucic
19	33.670	9570665	0.1789	338466	Arachidonic acid
20	34.343	1626479	0.0304	82980	tricosanoic
21	35.077	3191389	0.0597	178810	cis.docosanoic
22	35.705	1328713	0.0248	65031	cis.docosanoic
23	37.327	17310397	0.3236	449577	lignoceric
24	38.743	1692734	0.0316	70606	eicosapent
25	39.230	840723	0.0157	40398	nervonic
26	40.689	8140245	0.1522	340696	docosahexanoic
27	40.735	5310724	0.0993	345157	docosahexanoic
Total		5348582482	100.0000	14472147	

Fig. 5: standard curve of fatty acid compounds by GLC.

standard compound at a time of retention (25.221) minutes. Heneicosanoic acid at a time of retention (29.407) minutes and corresponds to the standard compound at a time of retention (29.737) minutes. Behenic acid at a time of retention (30.026) minutes and corresponds to the standard compound at a time of retention (30.333) minutes.



Peak Table – Channel 1

Peak#	Ret.Tem	Area	Area%	Height	Name
1	1.982	198745632	99.948	193547895	
2	3.568	598745	0.015	32568	
3	4.256	632563	0.013	65412	
4	5.093	589741	0.017	59874	
5	5.725	632147	0.019	56325	
6	6.292	987452	0.017	58741	
7	6.418	965874	0.012	45698	
8	7.079	845123	0.014	47856	
9	14.083	874123	0.016	56320	
10	15.039	654123	0.014	45896	
11	15.550	458963	0.012	23658	
12	17.781	874963	0.013	74896	
13	19.528	745896	0.014	41566	
14	25.085	896523	0.015	57896	
15	28.883	897452	0.016	74112	
16	29.407	965478	0.013	87452	
17	30.026	654123	0.012	98562	
18	30.618	985471	0.011	65986	
Total		2547965411	100.0000	199658745	

Fig. 6:. Curved fatty acid compounds for *Datura stramonium* by GLC.

Study the antioxidant efficacy of Alkaloids (hyoscine and atropine) and fatty acid compounds.

Alkaloids (hyoscine, and atropine)

Table 1 the results showed that alkaloids (hyoscine and atropine) separated from the datura plant gave the highest free-radical (DPPH) inhibitor (36%) and (40.8%) at concentration (400 µg/ml) and (500 µg/ml) respectively, The lowest free-radical inhibitor was (30) at concentration (200µg/ml). But the rate of inhibition of the free radical standard was superior in all concentrations and the highest inhibition (82.6%) at concentration (500 µg/ml) and the least inhibition (62.24%) at concentration (200 µg/ml). The results confirm that alkaloids oxidation is increasing inhibition of free radicals with increased concentration.

Fatty acid compounds

Table 1 and Fig. 8 shows that the fatty acids of the Datura plant recorded the highest free-radical inhibitory

Table 1: Concentration (µg/ml) and % of the standard sample and quaternary compounds hyoscine, atropine and fatty acid separated from the datura as antioxidants.

Concentration	200µg/ml	300µg/ml	400µg/ml	500µg/ml
Compound	Standard sample	Standard sample	Standard sample	Standard sample
	82.6%	77.7%	66.88%	62.54%
Hyoscine & atropine	40.8%	36%	%31.11	30%
Fatty acid	32.22%	30.88%	18.88%	%12.44

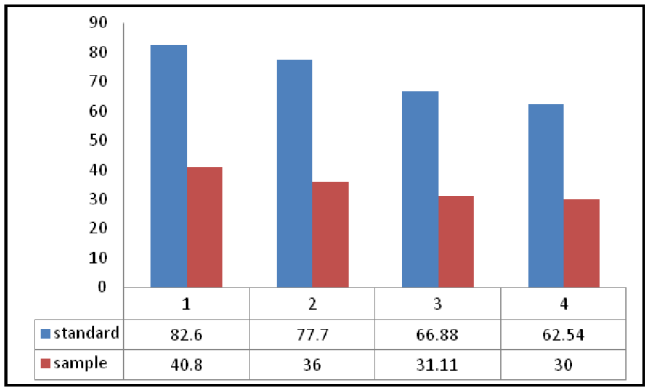


Fig. 7. Effect of hyoscine and atropine compounds on datura as antioxidant compared to ascorbic acid by DPPH method.

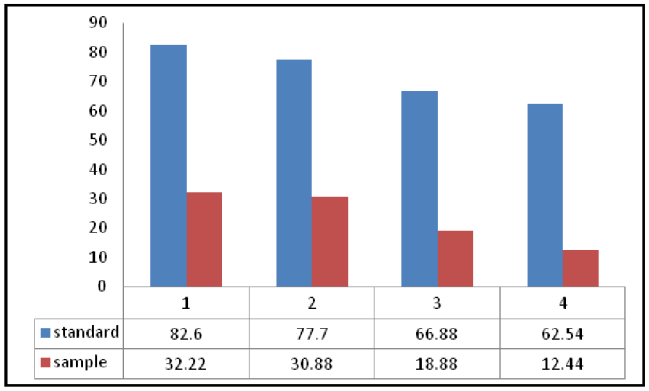


Fig. 8. Effect of fatty acids of Datura as antioxidant compared to ascorbic acid by DPPH method.

(DPPH) for concentration (500µg/ml) and Ration (32.22%), and The lowest inhibition ratio was (12.44%) for concentration (200µg/ml), as compared with the standard sample (L-Ascorbic acid) which was much better than fatty acids at the same concentration. The results show that alkaloids compounds of Datura plant are better than fatty acids in inhibiting free radicals, possibly because alkaloids compounds have more hydroxyl groups.

Conclusion

The HPLC technique diagnosis shows that *Datura stramonium* contains alkaloids (atropine and hyoscine). Also, the diagnosis of GLC technique proved that the plant contains fatty acids: (Caproic acid, Undecanoic acid, Pentadecanoic acid, Archidic acid, Heneicosanoic acid and Behenic acid). These compounds have a antioxidant effect in inhibiting free radicals, and the effect of compounds increases with their high concentration.

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