Identification and Characterization of Antioxidant and Bioactive Components of *Mirabilis Jalapa* and *Dracocephalum Moldavica* L. Plants

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Abstract

This study was conducted *Mirabilis Jalapa* and Dragonhead leaves and seeds to evaluate bioactive components. Carbohydrates represent the major component in the *Mirabilis.J* (75.20%).The content of Dragonhead Lipids and protein (28.5 – 23.9 %) was higher than that of *Mirabilis.J* (4.5 – 10.4%) respectively. *Mirabilis.J* and Dragonhead oil contains high amounts of unsaturated fatty acids. The unsaturated fatty acids of *Mirabilis.J* and Dragonhead oil were (79.21 and 88.55%) of total fatty acids respectively. The major unsaturated fatty acids in *Mirabilis.J* were linoleic acid (31.48%) followed by oleic (20.38%) and Gama linolenic (18.39%), while, total saturated fatty acids content was 17.87%. Dragonhead seed oil contains also major unsaturated fatty acids were Alfa-linolenic (51.88%) followed by linoleic (23.38%) and oleic acid (12.16%). Total phenolic and total flavonoid compounds, scavenging radical effect on 2, 2-diphenylpicrylhydrazyl (DPPH) and ABTS (2,2-azino-bis (3-ethlbenzthiazoline-3-sulfonic acid) radical-scavenging activity were investigated .The specific phenolic and flavonoid composition quantification for ethanolic extract were performed by HPLC. presence 21 phenolic compounds in dragonhead ethanolic extract. The highest quantities were , ellagic, benzoic, salycillic, ferulic, iso-ferulic and catechein while in *Mirabilis.J* ethanolic extract were pyrogallol, catechein, salycillic ,chlorogenic, ellagic and benzoic acid were found as the major phenols. Naringin, kamp.3, (2-p-comaroyl) glucose, luteolin.7glucose, hespirdin, rutin, apig.6arbinose.8.galactose, apigenin.-7 -o-neohes and quercetrin were found as the major flavonoids in dragonhead ethanolic extract however naringin, hespirdin, kamp.3, (2comaroyl) glucose and rutin were found as the major flavonoids in *Mirabilis jalapa*.

Key words: - *Mirabilis jalapa*, dragonhead, fatty acids, phenolic and flavonoid compounds.

Introduction

Mirabilis jalapa (family Nyctaginaceae) is a perennial herbal medicinal plant, has along traditional uses. Dried flowers used as a snuff for headaches, fungal infection and root decoction to wash wounds treat skin afflictions. It is used remedy for kidney stones and gallbladder chyluria (**Aoki *et al., 2008).***

The leaves are used as traditional folk medicine in the Brazil to treat inflammatory and painful diseases and as a laxative. The plant used for its antibacterial, antiviral, antifungal, antispasmodic, antitumor, diuretic, hydragogue, anti-oxidative, antimicrobial and anti-nociceptive actions (**Kumar *et al., 2010).***

Dragonhead (*Dracocephalum moldavica*) plants belong to family *Lamiaceae*. Also, it is recently introduced to Egypt during the last two decades it is a hardy plant native to regions from Eastern Europe to Siberia. The plant is widely used in folk medicine as pain killer and kidney complains .it is an easy and care free plant best massed in sunny or party shaded areas on well-drained soil (**Ismail, 2007**) .Dragonhead is used as painkiller for treatment of kidney complains, against toothache and colds as well as ant rheumatism. In addition, dragonhead plant is very attractive to honey bees and this may reduce their mortality when subjected to pesticide-contaminated ground cover. (**Dastmalchi *et al.*, 2007)**

Chemical analysis of *Mirabilis Jalapa leaves* showed that many active compounds including alpha-amyrins, beta-amyrins, beta–sitosterols, 2-carboxyarabinitol, campesterol, daucosterol, D-glucan, dopamine, hexaconsan1-ol, isobetanin, methyl labronisoflavone, olenolic acid, stigmasterol, tartaric acid, trigonelline, tryptophan and vulgaxanthin (**Tinoi *et al.*, 2006)**

Dracocephalum moldavica is a source of flavonoids and terpenoids such as luteolin, apigenine, oleanolic acid, ursolic acid, geranial, neral, limonene-10-al and rosmarinic acid and phenolic compounds contribute to antioxidant activity **Kakasy *et al.*, (2006) and (Fattahi *et al.*, 2013)**

Therefore, the aim of the present study is to assess chemical composition of *Mirabilis jalapa* and *Dracocephalum moldavica*, leaves and seeds. Fatty acids composition of seeds oil. Determination of *Mirabilis jalapa* and *Dracocephalum moldavica* protein subunits molecular weight by using SDS-PAGE. Determination of total phenolic, total flavonoids compounds and antioxidant activity were carried out.

Materials and Methods

Materials:

The *Mirabilis Jalapa* and dragonhead (seeds and leaves) were obtained from Farm Agricultural Research Station, Faculty of Agriculture at Moshtohor, Benha University. Samples were collected in 2017. All chemical used in these experiments were provided from Sigma and Aldrich chemical company of high quality and purity.

Analytical methods:

Moisture, total lipids, crude protein, ash, total carbohydrate and total fatty acids: were determined according to the method of the Association of Official Analytical Chemists **(A.O.A.C., 2005)**. The total fatty acids obtained from the oil samples were methylated by diazomethane and identified by gas liquid chromatography (GLC). Diazomethane was prepared from methylamine and urea as reported by **Vogel (1975)**.

Preparation of protein isolate from seeds meal:

-Extraction with alkaline solution, pH of protein precipitation and determination of protein subunits molecular weight:

Defatted *Mirabilis Jalapa* and Dragon head samples were extracted by using 0.02 N of sodium hydroxide according to the method described by **Melnychyn and Wolcott (1971).** The pH of protein precipitation was adjusted to cover pH ranges from 3.2 to 10.8. Each five grams of the sample was dispersed in the extracting solution for 15 min. The suspension was centrifuged at 10.000 rpm for 15 min. The supernatant was then transferred to a volumetric flask using distilled water and used for protein determination, according to of **Lowary *et al.* (1951).** Determination of protein subunits molecular weight by using polyacrylamide gel electrophoresis was preformed according to **Laemmli (1970)**.

Preparation of *Mirabilis jalapa* and dragonhead leaves extract:

The dried leaves was powdered mechanically and soaked with 80% ethanol (1:10) in brown bottles at room temperature (25-30^{o}C) in dark place for 7 day and mix gently every day. The mixture was filtrated by suction pump in Buchner funnel throw filter paper and concentrated to dryness using rotary evaporator and freeze dry.

Determination of total phenolic and flavonoids compounds

The concentration of total phenols in all extracts were measured by a UV spectrophotometer (SM1600UV-visSpectrphotometers, Azzota, USA), based on a colorimetric oxidation/reduction reaction as described by **Skerget *et al.* (2005).** Total flavonoids content was determined by the method of **Ordon *et al.* (2006).**

DPPH (2, 2-diphenylpicryhydrazyl) radical–scavening activity:

The electron donation ability of the obtained extracts was measured by bleaching of the purple colored solution of DPPH according to the method of **Hanato *et al.* (1988).**

ABTS (2, 2-azino-bis (3-ethlbenzthiazoline-3-sulfonic acid) radical-scavenging activity: $ABTS^{\bullet+}$ radical scavenging activity and adopted of extracts were measured by the $ABTS^{\bullet+}$ cation decolorization assay as described by **Re *et al.* (1999)**.

HPLC analysis:

The dried hydrolyzed ethanolic extracts were dissolved in HPLC grade methanol 1.0 mg/ml), filtered through sterile 0.22 µm Millipore filter and subjected to qualitative and quantitative analysis by using Shimadzu LC-IOA (Kyoto, Japan) HPLC instrument. **(Prakash, 2007)**

Result and Discussion

Chemical composition of Mirabilis. J and Dragonhead leaves and seeds

The chemical composition of *mirabilis. J* and dragonhead leaves and seeds were determined and reported in table (1).

Oil content in dragonhead leaves and seeds ranges from 3.14±0.59 to 28.50±0.59 g/100g dry weight basis whereas that carbohydrate represents the major components in dragonhead seeds and leaves ranged from 35.89±0.55 to 71.84±0.55 g/100g dry weight basis. The results showed that protein content ranged from 12.84±0.22 to 23.90±0.22 g/100g on dry weight basis in dragonhead leaves and seeds. Generally concluding Dragonhead seeds is considered as good source of protein and lipid.

From the obtained results it could be noticed that carbohydrate represents the major component in the *Mirabilis.J* (75.20%).The content of Dragonhead Lipids and protein (28.5 – 23.9 %) was higher than of *Mirabilis.J* (4.5 – 10.4%) respectively.

The accomplished results are in agreement with those reported by **Asima *et al.*, (2014), Hanczakakowsti *et al.*, (2009) and Dziki *et al.*, (2013**)

Table 1. Chemical composition of Dragonhead and *Mirabilis.J* leaves and seeds (g/100g dry weight basis).

Components	g/100g dry weight basis			
	Dragonhead		*Mirabilis.J*	
	Leaves	Seeds	Leaves	Seeds
Moisture	9.03±0.09	6.55±0.09	11.8±0.09	5.6±0.09
Lipids	3.14±0.59	28.50±0.59	2.53±0.26	4.5±0.26
Protein	12.84±0.22	23.90±0.22	6.86±0.12	10.4±0.12
Ash	3.15±0.03	5.16±0.03	3.42±0.05	4.3±0.05
***Carbohydrate**	71.84±0.55	35.89±0.55	75.29±0.69	75.2±0.69

*Total carbohydrate = 100 – (protein + fat + moisture + ash)

Effect of pH on protein isolation from *Mirabilis.J* and Dragonhead seeds:

Several experiments were carried out in order to establish the proper pH values required for *Mirabilis.J* and Dragonhead protein extraction. The obtained results are presented in Fig. (1) from these results it has shown that the maximum protein extraction was achieved at pH 11. On the other hand, results showed that on the acidic pH range, the percentage of the extracted protein was very lower than alkaline and reached its lowest amount at pH 4.0 – 5.0 (isoelectric point) in *Mirabilis.J* and Dragonhead respectively. However, at basic pH (11) the percentage of the extracted protein were found to be (94.12 – 94.23) from *Mirabilis.J* and Dragonhead respectively. The solubility of protein increases with increasing the acidity or alkalinity which might be attributed to the increase of repulsive electric forces induced by charges of some sign that might exist on protein molecules **(Ozcan, 2000)**.

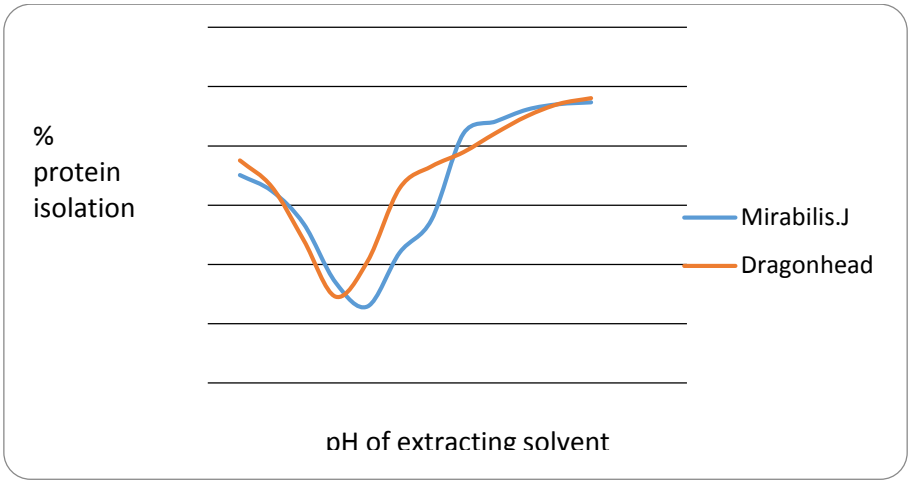

Fig. (1): Effect of pH on protein isolation from *Mirabilis.J* and Dragonhead seeds.

Determination of *Mirabilis.J* and Dragonhead protein subunits molecular weight by using SDS-PAGE:

Polyacrylamide Gel Electrophoresis in the presence of detergent Sodium Dodecyl Sulphate (SDS-PAGE) was used for determining the subunit molecular weights (M.W.) of protein extracted by alkaline solutions from *Mirabilis.J* and Dragonhead meal.

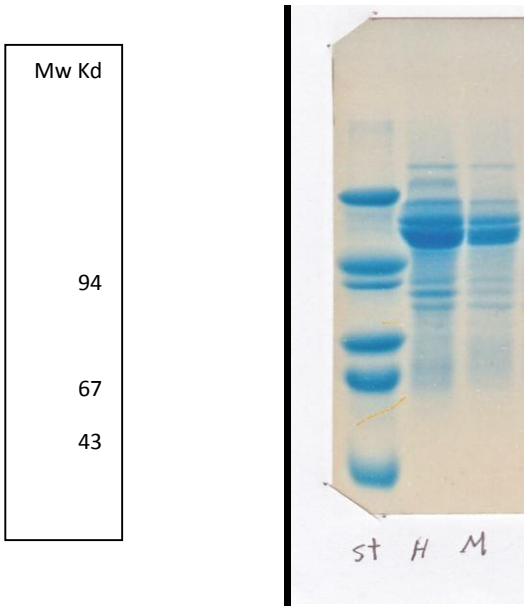

Fig. (2): SDS-PAGE pattern of protein extracts of Mirabilis.J and Dragonhead
St: standard protein
H: Dragonhead protein
M: Mirabilis.J protein

The obtained results show that the presence of 10 and 9 subunits respectively with molecules ranging from 95.000 to 33.000 KD in the Dragonhead protein and 94.000 to 33.000 in the *Mirabilis.J*. The achieved results are in agreement with those reported by **Rith *et al.*, 2010** found that the *Mirabilis.J* protein containing of 9 bands of MW86.7 to 13.5 KD.

Fatty acids composition of *Mirabilis.J* and Dragonhead seed oil:

Gas- liquid chromatography was used for the qualitative and quantitative determination of individual fatty acids methyl esters. Fatty acids composition of *Mirabilis.J* and Dragonhead seeds oil are presented in Table (2)

The obtained results showed that *Mirabilis.J* and Dragonhead oil contains high amounts of unsaturated fatty acids. The unsaturated fatty acids of *Mirabilis.J* and Dragonhead oil were (79.21 and 88.55%) of total fatty acid respectively.

The major component of unsaturated fatty acids in *Mirabilis.J* were linoleic acid (31.48%) followed by oleic (20.38%) and Gama linolenic (18.39%), while, total saturated fatty acids content was 17.87%. Among the saturated fatty acid, palmitic acid showed as a major percentage (11.39%) followed by stearic acids (3.82%) in *Mirabilis.J* seeds.

Dragonhead seed oil contained also major unsaturated fatty acids their value were Alfa-linolenic (51.88%), linoleic (23.38%) and oleic acid (12.16%). On the other hand two saturated fatty acid were identified palmitic acid as the major one which reached a value (5.97%) followed by stearic acids (3.14%).The obtained results are in agreement with those reported by **Patel and Patel (1985), Domokos *et al.*, (1994) and Hanczakowski *et al.*, (2009)** who found that dragonhead seeds are rich in oil ranged from (18-29%) this rich in unsaturated fatty acid about 90%.

Table 2. Fatty acids composition of Mirabilis.J and Dragonhead

Fatty acids	Mirabilis.J seeds oil %	Dragonhead seeds oil %
Lauric $C_{12:0}$	0.40	0.21
Tridecanoic $C_{13:0}$	0.98	0.55
Myristic $C_{14:0}$	0.49	0.37
Tetradecenoic $C_{14:1}$	0.22	0.17
Palimitic $C_{16:0}$	11.39	5.97
Palmitoleic $C_{16:1}$	0.14	0.38
Margaric $C_{17:0}$	0.07	0.15
Heptadecenoic $C_{17:1}$	0.02	0.09
Stearic $C_{18:0}$	3.82	3.14
Oleic $C_{18:1}$	20.38	12.16
Linoleic $C_{18:2}$	31.29	23.38
Gamma Linolenic $C_{18:3\ \omega6}$	18.39	---
Alfa Linolenic $C_{18:3\ \omega3}$	1.72	51.88
Archidic $C_{20:0}$	0.33	0.10
Godoleic $C_{20:1}$	3.68	0.49
Behenic $C_{22:0}$	0.39	0.04
Erucic acid $C_{22:1}$	3.37	--
Lignoceric $C_{24:0}$	---	0.20
Total saturated	17.87	10.73
Total unsaturated	79.21	88.55

Total Phenolic compound, total flavonoids and anti-radical activity of Dragon head and *Mirabilis .J* leaves extracts:

From the data presented in Table (3) it is clear that the ethanolic extract yield of Dragon head and Mirabilis .J were 22.98 % and 25.75 % respectively. However total phenolic content of Dragon head and *Mirabilis .J* leaves extract were519.13 and 326.75 mg/GAE extract.

Total flavonoids of dragonhead and *Mirabilis .J* leaves extract were 34.21 and 27.81 mg/GAE extract respectively. The accomplished results are good in agreement with those reported by **Mohamed *et al.*, (2010) and Mandegary e*t al.*, (2014).**

Antioxidant activity of the ethanolic extracts:

Ethanolic extracts from Dragonhead and Mirabilis .J leaves showed that strong scavenging activity against DPPH and ABTS radicals Table (3). From the abovementioned data it is clear that samples with low content phenolic compound have lower antioxidant activity. The antioxidant activity of phenolic compounds are to be largely determined by the number of hydroxyl groups on the aromatic ring. The higher number of hydroxyl groups, the greater expected antioxidant activity. These results are agreement with that reported by **Dodonné *et al.*, (2009), Zachariah *et al.*, (2011) and Gill-Miron., (2016).** Data presented in table (3) shows antioxidant activity of dragonhead was higher than mirabilis in leaves extracts in both DPPH 87.64%, 70.09% and ABTS 82.35%, 65.80% respectively.

Table 3. Ethanolic extract yield, total phenolic, total flavonoid and antiradical activities of Dragonhead and Mirabilis .J leaves extract:

Parameter / Material	Total extract %	Total phenolic mg / g extract	Total flavonoids mg / g extract	% Antiradical activity	
				DPPH	ABTS
Dragonhead leaves	**22.98±1.45**	**519.13±0.14**	**34.21±0.95**	**87.64±1.51**	**82.35±1.17**
Mirabilis. J Leaves	**25.75±1.34**	**326.75±0.19**	**27.81±1.17**	**70.09±1.43**	**65.80±1.31**

Identification of antioxidant components in Dragonhead and Mirabilis. J leaves ethanolic extract by HPLC:

Data presented in Table (4 and 5) showed the chemical constituents of the ethanolic extract of dragonhead and Mirabilis. J leaves.

The results in table (4) revealed the presence 21 phenolic compounds in dragonhead ethanolic extract. The highest quantities were, ellagic (1443.74mg/100g), benzoic (577.33mg/100g), salycillic (576.77mg/100g), ferulic (359.9 mg/100g), iso-ferulic (233.91mg/100g), catechein (195.02mg/100g), P-OH-benzoic (179.45 mg/100g), caffeine (175.29mg/100g), and pyrogallol (115.53mg/100g).

The results in table (5) revealed the presence 15 flavonoid compounds in dragonhead leaves ethanolic extract. The highest quantities were naringin (3331.73 mg / 100 g) , kamp.3, (2-p-comaroyl) glucose (2685.11 mg / 100 g) , luteolin.7 glucose (1582.36 mg / 100g) hespirdin (1395.9mg/100g) , rutin (394.70mg / 100 g) , Apig.6-arbinose.8.galactose(342.18mg/100g), apigenin.-7 -o-neohes (316.9mg/100g) and quercetrin (289.90mg/100g) .

The results in table (4) revealed the presence 21 phenolic compound in *Mirabilis.J* ethanolic extract .The highest quantities were pyrogallol (764.91mg/100g), catechein (542.38mg/100g), salycillic (149.07 mg/100g), chlorogenic (89.68 mg/100g), ellagic (73.89 mg/100g) and benzoic (73.10mg/100g).

The results in table (5) revealed the presence 15 flavonoid compounds in *Mirabilis.J* leaves ethanolic extract. The highest quantities were naringin (470.09mg/100g), hesperidin (335.18mg/100g), kamp.3, (2comaroyl) glucose (197.76mg/100g) and rutin (172.57mg/100g). The results are good in agreement with those reported by **Saeidnia *et al*., (2005), Dastmalchi *et al.*, (2007),Popova *et al* .,(2008), Mohamed *et al*., (2010), wang and Dai, (2012), and Liqi *et al*., (2016).**

Table 4. Phenolic compounds of dragon head and Mirabilis J. leaves ethanolic extracts analyzed by HPLC

	phenolic compounds	Dragon head *mg/100g*	Mirabilis .J *mg/100g*
1	Gallic	13.73	7.45
2	Pyrogallol	115.53	764.91
3	4-aminobenzoic	13.62	23.05
4	Protocatchuic	79.65	58.27
5	Catechein	195.02	542.38
6	Chlorogenic	90.88	89.68
7	Catechol	108.53	64.99
8	Caffeine	175.29	58.19
9	p-OH-benzoic	179.45	10.83
10	Caffeic	28.11	5.70
11	Vanillic	27.23	17.89
12	P-coumaric	72.72	2.24
13	Ferulic	359.90	12.83
14	Ios-ferulic	233.91	13.95
15	Ellagic	1443.74	73.89
16	Alpha-coumaric	26.96	0.60
17	Benzoic	577.33	73.10
18	Salycillic	576.77	149.07
19	3,4,5methoxycinnamic	47.63	15.29
20	Coumarin	67.95	9.65
21	Cinnamic	10.93	5.72

Table 5. Flavonoids compounds of dragon head and Mirabilis J. leaves ethanolic extracts analyzed by HPLC

	Flavonoids	dragon head *mg/100g*	Mirabilis .J *mg/100g*
1	A Pig.6-arbinose 8- galactose	342.18	42.59
2	A Pig.6- rhamnose 8- glucose	173.12	52.73
3	Luteolin 7-glucose	1582.36	64.80
4	Naringin	3331.73	470.09
5	Rutin	394.70	172.57
6	Hespirdin	1395.59	335.18
7	Apigenin.7 o-neohes	316.90	16.13
8	Quercetrin	289.90	66.14
9	Quercetin	24.57	9.89
10	Kamp.3(2-p-comaroyl)glucose	2685.11	197.76
11	Naringenin	119.20	43.38
12	Kampferol	9.45	4.61
13	Acacetin neo.rutinoside	132.02	20.65
14	Hespirtin	114.28	29.85
15	Apegnin	75.84	11.46

Conclusion

In conclusion, the present study confirm that leaves and seeds of dragon head and *Mirabilis .J* plant are rich source in some important phytochemical compounds. The ethanolic extract is highly valuable source of natural antioxidant and showed the presences of different bioactive compounds with high antioxidant activity. Further research should be addressed on the application of using leaves extracts of dragon head and Mirabilis .J as natural agent protect against per-oxidative damage in living systems related to diabetic , aging and carcinogenesis

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توصيف وتعريف المركبات البيولوجية النشطة والمضادة للاكسدة فى نباتى رأس التنين وشب الليل

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تهدف هذة الدراسة الى تقدير التركيب الكيميائى لبذور وأوراق نباتى رأس التنين وشب الليل ، تقدير نسبة الاحماض الدهنية ، تقدير الوزن الجزئيى للبروتين ، أستخلاص المواد الفعالة بواسطة كحول الايثايل 80% تقدير المركبات الفينولية والفلافونيدات الكلية فى المستخلص وكذلك تقدير نشاط المواد المضادة للاكسدة و فصل وتفريد المركبات الفعالة .أوضحت النتائج ان بذور نباتى راس التنين وشب الليل كان محتواهم من الرطوبة (5.60–6.55 %) ، نسبة الزيت (28.5– 4.5%) ، نسبة البروتين (10.4–23.9 %) ، نسبة الرماد (4.3–5.16 %) ونسبة الكربوهيدرات الكلية (75.20–35.89 %) على التوالى . أوضحت نتائج التحليل الكروماتوجرافى الغازى للاحماض الدهنية لزيت بذورنباتى راس التنين وشب الليل على أنها تحتوى على نسبة عالية من الاحماض الدهنية الغير مشبعة (88.55 –79.21 %)0أوضحت النتائج الى ان المستخلص الكحولى لكل من اوراق نباتي رأس التنين وشب الليل يحتوى على نسبة عالية من المركبات الفينولية تتراوح مابين (519.13 –326.75) مللجرام /جرام مستخلص كحامض جاليك على التوالي .أوضحت النتائج ان المستخلص الكحولى لكل من اوراق نباتي رأس التنين وشب الليل يحتوى على نسبة عالية من المركبات الفلافونيدية تتراوح مابين .(34.21 –27.81) مللجرام /جرام مستخلص كحامض جاليك على التوالي .كما ان تلك المستخلصات لها نشاط كمضاد أكسده ضد ماده داي فينيل بيكريل هيدرازيل مابين وضد ABTS . وبتفريد تلك المستخلصات على جهاز التحليل الكروماتوجرافى السائل وجد انها تحتوى على 21مركب فينولى فى مستخلص راس التنين اكثرها تركيزا كالتالى (حمض الالجيك 1443.74مللجرام/100جرام ,حمض البنزويك 577.33مللجرام/100جرام ,حمض السالسليك 576.77 مللجرام /100جرام وحمض الفيروليك 359.9 مللجرام/100جرام) اما بالنسبه لمستخلص شب الليل فكان يحتوى ايضا على 21مركب فينولى اكثرها تركيزا كالتالى (البيروجالول بتركيز 764.91 مللجرام/100جرام ,الكاتشين 542.38 مللجرام/100جرام ,حمض السالسليك 149.07 مللجرام/100جرام والكلوروجينيك 89.68 مللجرام/100جرام)0وبتفريد تلك المستخلصات على جهاز التحليل الكروماتوجرافى السائل وجد انها تحتوى على 15مركب فلافونويد فى مستخلص راس التنين كالتالى (النارنجين 3331.37مللجرام/100جرام , كامب –3 – جلوكوز 2685.11 مللجرام/100جرام والليتيونين–7– جلوكوز 1582.36 مللجرام/100جرام) اما بالنسبه لمستخلص شب الليل فكان يحتوى ايضا على 15مركب فلافونويد كالتالى (النارنجين 470.09 مللجرام/100جرام ,والهسبريدين 335.18 مللجرام/100جرام ,كامب –3– جلوكوز 197.76 مللجرام/100جرام والروتين 172.57 مللجرام/100جرام).

الكلمات الدالة:– شب الليل ، رأس التنين ، الاحماض الدهنية ، المركبات الفينولية والفلافونيدية