JCBPS; Section B; August 2018 – October 2018, Vol. 8, No. 4; 867-869 E- ISSN: 2249 –1929

[DOI: 10.24214/jcbps.B.8.4.85769.]

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Evaluation of antibacterial activity, antioxidant properties of *Cynara Scolymus* L. aqueous leaf and pulp extracts and their effect on streptozotocin-induced diabetic rats

Hanan M. EL-Ghandour, Ola A. Wahdan and Ghadir A. El-Chaghaby

Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

Received: 24 July 2018**; Revised:** 13 August 2018; **Accepted:** 20 August 2018

Abstract: *Cynara Scolymus L.* (Artichoke) is appreciated as a medicinal plant possessing health benefits. Aqueous extracts of artichoke leaf and pulp were prepared and tested for antibacterial activity against four Gram-negative bacteria (*Escherichia coli, Nisseria gonorrhoeae, pseudomonas aeruqinosa and Salmonella sp.*) and five Gram-positive bacteria (*Bacillus cereus, Listeria monocytogenes, Bacillus subtilis, Streptococcus faecalis* and *Staphylococcus aureus*). Data revealed that aqueous pulp extract had moderate antibacterial activity against tested bacteria except *Salmonella sp.* for which the extract did not show any inhibition activity. Whereas, the aqueous leaf extract showed better activity against tested Gram-positive bacteria except for *Bacillus cereus* species. Also, leaf extract exhibited activity against tested Gram-negative bacteria except *Salmonella sp.* The total antioxidant capacities of *Cynara* leaf and pulp aqueous extracts′ valued 151.15 and 63.18 ppm ascorbic acid equivalent, respectively. Gas chromatographic/ mass analysis ensured the existence of more than 40 bioactive compounds in the extracts. Biological experiment was conducted on streptozotocin induced diabetic rats. Diabetes is associated with inflammation and generation of excessive free radical. Erythrocyte Sedimentation Rate (ESR) and C-Reactive ProteinC - reactive protein (CRP) tests were monitored as inflammation biomarkers. Supplementation with *Cynara* aqueous extracts led to decreased ESR levels indicating minimization of inflammatory condition. Blood glucose and ALT

levels increased significantly (p=0.05) in diabetic group, while CRP was not affected and negative results were obtained in all treated groups. In conclusion, the results showed that both extracts ameliorated ESR levels reflecting the fact that they improve health status by vanishing the inflammatory and oxidative stress conditions associated with hyperglycemia.

Keywords: Cynara extracts; antibacterial screening; antioxidant activity; diabetes; inflammation biomarkers.

INTRODUCTION

Botanical antioxidants can be consumed for longer periods of time without any adverse effects. Dietary flavonoids have anti-carcinogenic and anti-inflammatory properties[1].*Cynara Scolymus L.* is a plant packed with many bioactive compounds and nutrients that could increase the antioxidant status and consequently promote health. Previous investigations have proven that artichoke extract has strong antioxidant efficiency on several test systems [2].Diabetes mellitus is a prevalent disease that is related to inflammatory condition[3]. According to Kalsait *et al.*[4] C-reactive protein (CRP) is a useful biomarker of injury and inflammation. Streptozotocin induced diabetes provided a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia [5].

Diabetes related symptoms comprising oxidative stress and inflammation could be controlled by the consumption of natural antioxidants from plant extracts such as *Cynara Scolymus L*. Egypt is one of the top producers of *Cynara* amounted to 387.704 tons annually[6]. Artichoke processing generates waste, consisting mainly of the outer and inner bracts, representing ≈70% of the weight of its head[7]. It is thus of environmental interest to find out possible uses for this high amount of waste in order to minimize pollution. In this respect, the present study aims to (1) determine the antibacterial activity of *Cynara* leaf and pulp extracts′ against nine bacterial strains, (2) investigate the antioxidant properties of both extracts and to identify their active compounds and (3) to evaluate the impact of *Cynara* extracts′ on minimizing the oxidative stress induced by streptozotocin in male rats.

MATERIAL AND METHODS

Preparation of aqueous Cynara leaf and pulp extracts′: One gram of *Cynara* dried leaf or fresh pulp was immersed in one hundred milliliter boiled water for 15 min. and filtered [8]. The extracts were prepared fresh daily and administered *ad-libitum* to be the sole source of fluid.

Antibactrial activity: Aqueous extracts will be screened for antibacterial activity against nine bacterial strains: four Gram-negative bacteria (*E. coli, Nisseria gonorrheae , pseudomonas aeruqinos and salmonella sp.*) and five Gram-positive bacteria (*Bacillus cereus, Listeria monocytogenes, Bacillus subtilis, Streptococcus faecalis* and *Staphylococcus aureus*). The tested bacteria were grown in buffered peptone water (pH 7.2) and incubated for 24 h at 37°C to achieve viable cell count of 10^8 cfu/ml.

The antibacterial activity of the extracts was carried out according to William[9]. Mueller-Hinton agar was the selected media for preparing the test plates. 100µl of the microbial suspension was taken and spread onto Mueller-Hinton agar. 500 µl of extracts were placed in holes in the agar layer seeded with the above mentioned bacteria. Negative control was included using ethanol 70% as described by Liviu *et al.*[10].The inoculated plates were incubated at 37°C for 48 h. All tests were performed in triplicate.

Antioxidant assays and phytochemical screening of Cynara leaf and pulp extracts:′ Total antioxidant capacity (TAC) was determined according to the procedure described by Prieto *et al.* [11]. Total phenolics (TP), total flavonoids (TF) and Ferric reducing antioxidant power (FRAP) were determined according to Singleton *et al.*[12], Zhishen *et al.*[13] andOyaizu[14], respectively. Qualitative determination of tannins, glycosides, alkaloids and saponins was conducted following the procedures described by Harborne[15].

Gas Chromatography spectrometry analysis of Cynara aqueous extracts: Identification of the various compounds present in the extracts was performed using GC-MS technique.

Chemicals: Streptozotocin mixed anomers was obtained from Sigma-Aldrich Company (Sigma No. S0130) with molecular formula $C_8H_{15}N_3O_7$ and molecular weight 265.2.

Citric acid anhydrous ($C_6H_8O_7$, M.Wt. =192) was purchased from Sd fine chemical limited (SDFCl). Trisodium citrate anhydrous ($C_6H_5O_7Na_3$, M.Wt. = 258) was purchased from Adwic company.

Reagents : 0.1 M citric acid was prepared by dissolving 1.92 g in 100 ml deionized water (Solution A).0.1 M sodium citrate was prepared by dissolving 2.58 g in 100 ml deionized water (Solution B).

Preparation of citrate buffer pH 4.4: 0.1 M citrate buffer pH 4.4 was prepared according to Stoll and Blanchard[16] by mixing 49.5 ml of solution A with 50.5 ml of solution B. STZ-buffer solution was freshly prepared before injection to avoid degradation of STZ.

Biological experiment design: Thirty six male albino rats weighing 150-160 gm were acclimatized for three days and fed on the standard diet AIN 76[17] and supplied water *ad-libitum*.

Rats were divided into six groups:

Group A served as negative control, Group B was set as positive control and rats were injected with a single interperitonial dose of STZ (50 mg/kg bw) in 0.1 M citrate buffer of pH 4.4.

Groups C and D took leaf and pulp extracts′ as the sole source of fluid, respectively. Groups E and F were injected with a single interperitonial dose of STZ (50 mg/kg bw) and administered leaf (CLE) and pulp (CPE) extracts′ as the sole source of fluid for six weeks. At the end of the experiment, blood samples were collected, centrifuged at 4000 rpm for five minutes, serum was kept refrigerated till subsequent analysis of urea [18], AST&ALT[19], total protein[20] and albumin[21]. Hemoglobin was determined using SYSMEX, XT-2000i VA instrument.

Inflammatory biomarkers: Qualitative screening of C - reactive protein in serum was detected using latex agglutination test kit supplied from Egyptian Company for Biotechnology (Spectrum). Erythrocyte Sedimentation Rate, the rate at which red blood cells sediment in a period of one hour, was determined according to Westergren[22].

RESULTS AND DISCUSSION

Tables **(1-a and 1-b**) show the antibacterial activity results of Cynara pulp and leaf aqueous extracts given as inhibition zone diameter (mm). Data revealed that aqueous pulp extract had moderate antibacterial activ ity against Gram-positive bacteria *(Bacillus subtilis, Streptococcus faecalis* and *Staphylococcus aureus)* w ith inhibition zones of 4, 4 and 4 mm diameters, respectively. Inhibition zones of pulp extract against Gra m-negative bacteria (*E. coli, Nisseria gonorrheae and pseudomonas aeruqinosa* were found to be 4, 6 and 4 mm, respectively. On the other hand, the extract did not show any activity against *Salmonella sp*.

Table (1-a): Antibacterial activity of leaf and pulp extracts against Gram-positive bacteria

	Bacillus cereus	***Listeria monocytogenes***	***Bacillus subtilis***	***Streptococcus faecalis***	***Staphylococcus aureus***
Aqueous pulp extract	**0.0**	**0.0**	**4.0**	**4.0**	**4.0**
Aqueous leaf extract	**0.0**	**9**	**7.0**	**7.0**	**6.0**

Average of triplicate determinations of inhibition zone diameter (mm).

Table (1-b): Antibacterial activity of leaf and pulp extracts against Gram-negative bacteria

	Neisseria gonorrhea	***Pseudomonas aeruginosa***	***E. coli***	***Salmonella sp.***
Aqueous pulp extract	**6.0**	**4.0**	**4.0**	**0.0**
Aqueous leaf extract	**9.0**	**7.0**	**6.0**	**0.0**

Average of triplicate determinations of inhibition zone diameter (mm).

Aqueous leaf extract had remarkable activity against Gram-positive bacteria *(Listeria monocytogenes*, *Bac illus subtilis*, *Streptococcus faecalis* and *Staphylococcus aureus)* with inhibition zones of 9, 7, 7 and 6 mm, respectively. Yet, no antibacterial activity was noticed for *Bacillus cereus* species. Leaf extract exh ibited activity against Gram-negative bacteria (*E. coli*, *Nisseria gonorrheae and pseudomonas aeruqinos)* with inhibition zones of 6, 9 and 7 mm, respectively. While, negative result was obtained for *Salmonella s p.* The antibacterial activity of leaf extracts could be attributed to the presence of phenols, flavonoids and a lkaloids that mediated antimicrobial potency by complexing with cell wall and inactivated enzymes as cite d by Cowan [23].

CLE and CPE had total antioxidant capacities (TAC) of 151.15 and 63.18 ppm as ascorbic acid equivalent , respectively. Total flavonoids (TF) valued 19.09 and 2.91 ppm as quercetin equivalent, respectively. Tot al phenols (TP) accounted to 75.4 and 6.67 ppm as gallic acid equivalent, respectively **(Table 2).**

Results of FRAP are shown in **figures 1 and 2**. For each extract four different concentrations (4, 6, 8 and 10 mg/ml) were used. The ferric reducing power of a sample is a measure for the presence of reductants su ch as antioxidant substances in the samples which causes the reduction of the Fe^{3+} to Fe^{2+} form. As the red ucing power increases the absorbance will increase. Our results indicate that CLE and CPE have potential FRAP and within the same concentration it can be observed that the FRAP of CLE is higher than that of C PE. This is in accordance with the results of TAC, TF and TP.

Qualitative detection of extracts ensured the presence of tannins and saponins **(Table 3)**. Whereas, both ex tracts were free from glycosides and alkaloids. Phytochemical screening is a valuable step in the detection of biological active compounds.

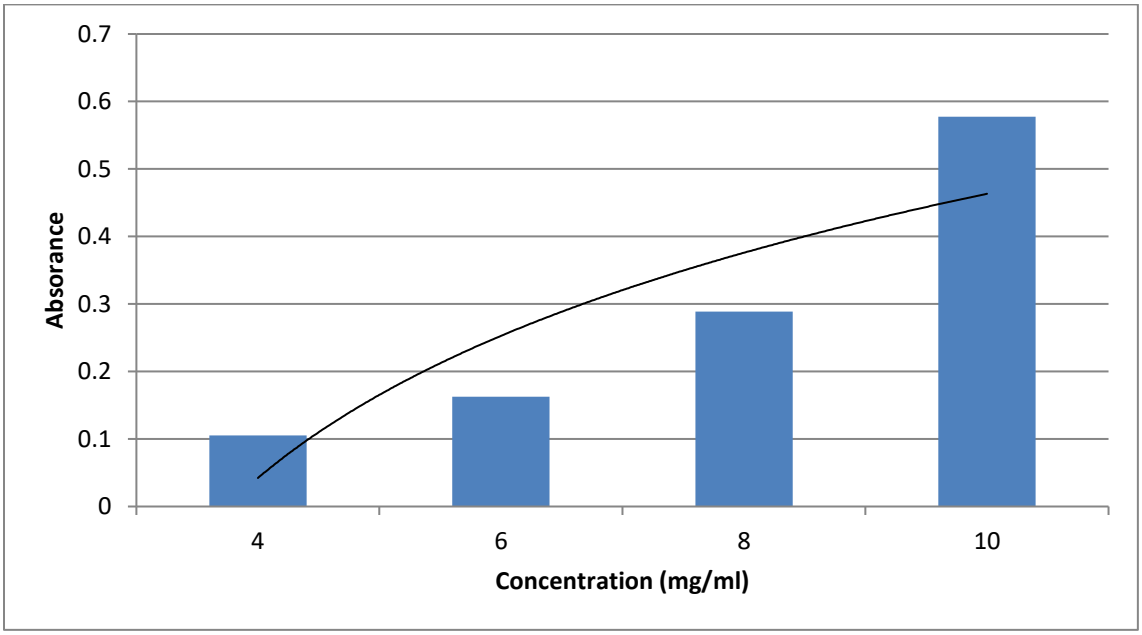

Fig. (1): FRAP of aqueous Cynara leaf extract

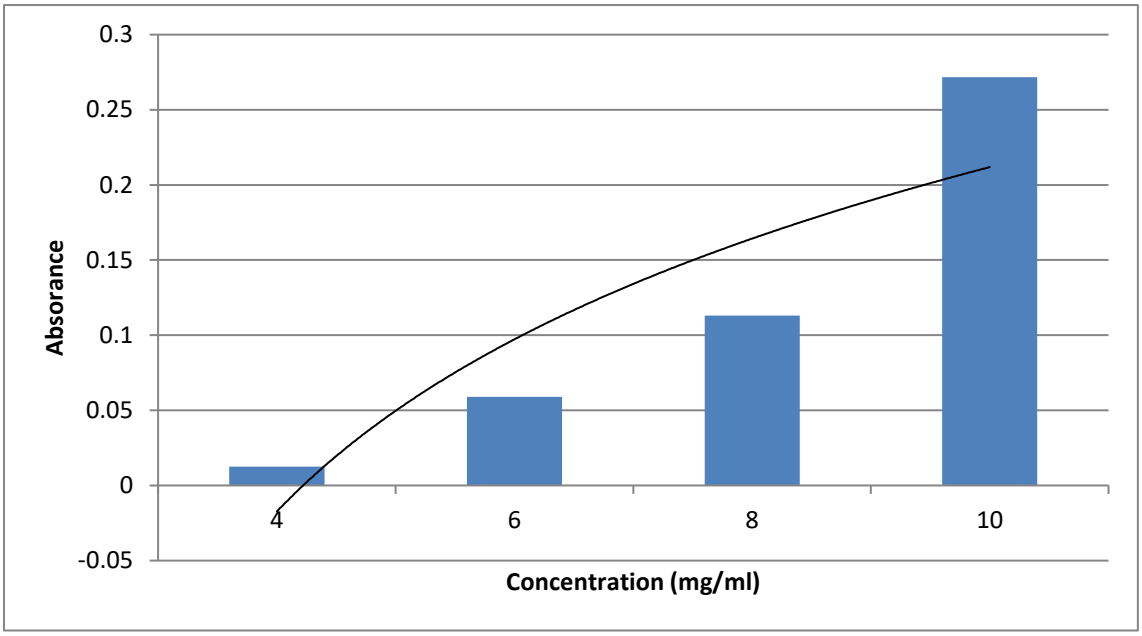

Fig. (2): FRAP of aqueous Cynara pulp extract

Table (2): Phytochemical analysis of Cynara leaf and pulp extracts′

Extract / Chemical analysis	Leaf extract	Pulp extract
Total antioxidant capacity (ppm as TAA)	151.15	63.18
Total flavonoids (ppm as quercetin equivalent)	19.09	2.91
Total phenols (ppm as gallic acid equivalent)	75.4	6.68

Results were expressed as average of three determinations.

Table (3): Qualitative determination of glycosides, tannins, saponins and alkaloids in leaf and pulp extracts′

Extract / Tested parameters	Leaf extract	Pulp extract
Glycosides	Negative	Negative
Tannins	Positive	Positive
Saponins	Positive	Positive
Alkaloids	Negative	Negative

As shown in Tables **(4 and 5),** GC-MS analysis of Cynara pulp and leaf extracts′ ensured the presence of 41 and 42 bioactive compounds, respectively. Thirty one compounds are commonly found in both extracts. Nine major compounds were exclusively identified in pulp extract: tetra-O-methylfisetin, coniferyl aldehyde, phenol, colchicine, levallorphan, squalane and astaxanthin.

Ten active phytochemicals were uniquely found in the leaf extract: salbutamol, esculetin, flavone, p-hydroxybenzoic acid, malvidin-3-glucoside, scopoletin, p-cresol, hydroquinone and cyaniding cation.As seen in **Tables (6-7),** a significant increase (p=0.05) in ALT, ESR and glucose levels was noticed in diabetic rat group in comparison with negative control. High ESR level indicated an inflammation state as cited by Malcolm[24].Results ensured that diabetes mellitus didn't provoke any change in AST, hemoglobin, urea, total protein and albumin levels. CRP was tested as a marker assessing levels of inflammation. Results registered negative results for all treated groups, reflecting the fact that diabetes mellitus did not provoke any change in CRP levels. Administration of *Cynara* leaf and pulp extracts′ led to decrease in ALT and ESR levels. Pulp extract aid in decreasing glucose level by 35%, while, leaf extract contributed slightly in the reduction of glucose by 10%.

Table (4): GC-MS chromatogram of aqueous pulp extract

RT	Name	Area sum (%)
3.238	4-Methylcatechol	46.91
4.841	Cumaldehyde	6.42
5.58	3,5-di-t-Butylcatechol	2.5
5.516	Caffeic acid dimethyl ether	1.37
6.893	6-Hydroxyflavone	1.02
7.26	Phenol, 2,4-diisopropyl-	2.05
7.376	Ferulic acid	0.61
7.813	Gallic acid	9
9.034	Vanillic acid	0.62
9.309	Phenol, 4-tert-butyl	0.67
9.508	β-Resorcylic acid	0.94
9.572	Gentisic acid	2.82
10.424	Syringic acid	0.61
10.726	Phloroglucinol	0.52
10.974	Salicylic acid	0.61
11.126	Tetra-O-methylfisetin	0.52
11.227	Cinnamic acid	3.04
11.478	Protocatechuic acid	0.57
12.162	Coniferyl aldehyde	0.9
12.412	3,4-Dihydroxymandelic acid	0.64
12.696	2-Hydroxyphenylacetic acid	1.91
12.861	Benzoic acid, 2,6-dihydroxy	0.51
13.374	Vitamin E	0.56
13.438	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	0.47
13.683	3-Hydroxy-4-methoxycinnamic acid	0.49
13.991	3,4-Dihydroxyphenyl glycol	1.15
14.074	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	0.58
14.605	2,5-Di-tert-butylquinone	0.51
14.828	Flavone, 3,5,7-trimethoxy-	0.68
15.112	Vitamin B6	0.77
15.195	9-cis-Retinoic acid	0.56
15.876	Colchicine	0.57
16.083	Levallorphan	0.6
16.147	2,6-di-t-Butyl-4-hydroxymethylphenol	0.71
16.236	4-tert-Pentylphenol	0.63
16.651	Squalane	2.84
17.342	Propyl gallate	0.71

18.838	Thymol	0.67
22.073	Phenol, 2,6-di-tert-butyl-	0.68
22.689	Lycopene	1.36
24.031	Astaxanthin	0.7

Table (5): GC-MS chromatogram of aqueous leaf extract

RT	Name	Area sum (%)
3.399	4-Methylcatechol	31.33
4.688	Cumaldehyde	7.58
4.762	Salbutamol	7.42
5.366	3,5-di-t-Butylcatechol	4.39
5.583	Caffeic acid dimethyl ether	0.94
6.872	6-Hydroxyflavone	1.43
7.177	Esculetin	1.22
7.266	Phenol, 2,4-diisopropyl-	5.18
7.348	Ferulic acid	0.81
7.803	Gallic acid	9.95
9.016	Vanillic acid	0.81
9.095	Flavone, 4',5,7-trimethoxy-	0.57
9.297	Phenol, 4-tert-butyl	0.66
9.502	β-Resorcylic acid	0.73
9.572	Gentisic acid	4.51
9.883	p-Hydroxybenzoic acid	0.54
10.421	Syringic acid	0.32
10.72	Phloroglucinol	0.75
10.964	Salicylic acid	0.39
11.224	Cinnamic acid	4.43
11.474	Protocatechuic acid	0.72
11.826	Malvidin-3-glucoside	0.51
12.027	Scopoletin	0.42
12.152	Phytol	0.69
12.412	3,4-Dihydroxymandelic acid	0.46
12.69	2-Hydroxyphenylacetic acid	2.98
12.852	Benzoic acid, 2,6-dihydroxy	0.7
13.374	Vitamin E	0.36
13.454	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	0.55
13.689	3-Hydroxy-4-methoxycinnamic acid	0.39
13.991	3,4-Dihydroxyphenyl glycol	1.97
14.08	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	0.4

14.452	Hydroquinone, 2,5-di-tert-pentyl-	0.5
14.611	2,5-Di-tert-butylquinone	0.59
14.834	Flavone, 3,5,7-trimethoxy-	0.56
15.112	Vitamin B6	1.06
15.185	9-cis-Retinoic acid	0.35
15.665	Cyanidin cation	0.43
16.147	2,6-di-t-Butyl-4-hydroxymethylphenol	0.7
17.332	Propyl gallate	0.51
18.835	Thymol	0.82
22.735	Lycopene	0.38

Table (6): Blood parameters of treated groups

Group / Tested parameters	**Negative control (Group A)**	**Positive control (Group B)**	**LE Control (Group C)**	**PE Control (Group D)**	**STZ and LE (Group E)S**	**STZ and PE (Group F**
ALT (mg/dl)	55±7	97±2*	50±4	46±6	70±0.9	65±2
AST (mg/dl)	107±10	111±16	97±5	101±8	108±17	110±7
Hb	15.8±0.65	15.86±0.56	15.43±0.35	14.86±0.25	16.1±0.4	15.13±0.6
Urea	55.75±4.03	69.5±11.0	56±3.3	55.5±2	64±5	67±8
Total protein	6.82±0.35	5.97±0.27	6.8±0.3	6.7±0.4	6.02±0.45	6.0±0.21
Albumin	3.4±0.37	3.0±0.35	3.4±0.37	3.4±0.37	3.25±0.46	3.1±0.25
Glucose	109±15	573±10*	104±13	101±11	517±9*	370±6*

*** Significant difference (p=0.05) in comparison with negative control**

Table (7): Inflammation biomarkers of different treated groups

Group / Tested parameters	**Negative control (Group A)**	**Positive control (Group B)**	**LE Control (Group C)**	**PE Control (Group D)**	**STZ and LE (Group E)S**	**STZ and PE (Group F**
ESR (mm/hr)	1±0.04	2±0.1*	1.08±0.02	1.05±0.03	1.3±0.03	1.29±0.02
CRP	Negative	Negative	Negative	Negative	Negative	Negative

*** Significant difference (p=0.05) in comparison with negative control**

The antioxidant and anti-inflammatory activities of the extracts contributed in minimizing the oxidative st ress and inflammation status related to diabetes mellitus as proved by decreasing ESR levels. Dietary antiox

idants reduced oxidative stress and improved defense mechanism [25].

GC-MS technique identified the phytoconstituents in order to relate their presence with activities of *Cynara Scolymus L* extracts.The extracts were of highest therapeutic efficacy possessing majority of phytochemical classes comprising phenolic compounds, flavonoids, carotenoids and coumarin.

Fisetin is a bioactive flavonol molecule found in fruits and vegetables that had antioxidant activity [26]. Flavonoids had significant biological activities such as antioxidant, anticarcinogenic, anti-inflammatory, antibacterial, immune-stimulating, and antiviral [27]. Flavonoids scavenge free radicals implicated in the development of diabetes mellitus and carcinogenesis [28]. Hyperglycemia can cause inflammation of the blood vessels and lead to serious damage. These inflammatory processes are inhibited by treatment with fisetin possibly via inhibition of the HMGB1 signaling pathway.

Coniferyl aldehyde is a phenolic compound known to have antioxidant capacity [29].

Phenol (2-hydroxyethyl) known as tyrosol is a natural phenolic[30] that restore intracellular antioxidant defenses [31].

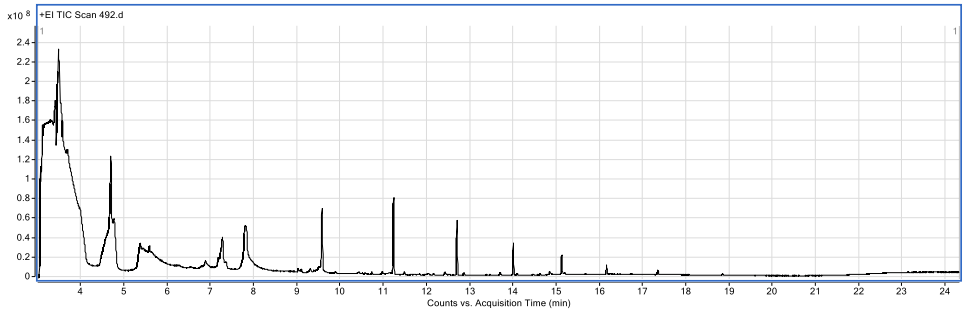

Fig. (3): GC-MS chromatogram of aqueous leaf extract

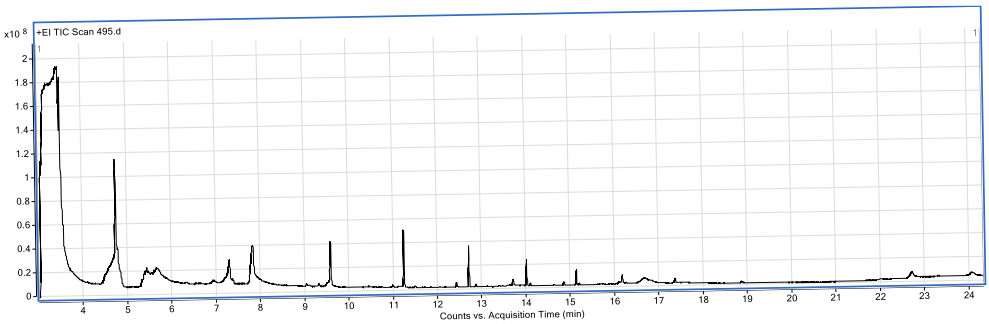

Fig. (4): GC-MS chromatogram of aqueous pulp extract

Colchicine elevated total antioxidant capacity in patients with knee osteoarthritis [32]. Co-administration of colchicine ameliorated both acute [33] and chronic liver damage [34] in rats. Rojkind [35] reported that colchicine possessed anti-inflammatory action. Charlton-Menys and Durrington [36] informed that squalene modulated antioxidative activity.Astaxanthin, belongs to the carotenoid family, has potential health-promoting effects

in the prevention and treatment of cancer, inflammatory conditions, diabetes and liver diseases as reviewed by Yuan *et al.*[37].

Astaxanthin had no pro-oxidant activity and its main sight of action is on/in the cell membrane [38]. Salbutamol detected in*Cynara*leaf extract was reported to play a remarkable role in preventing inflammation by stimulating β-2 adrenergic receptors and vanishing ROS generation in rats [39]. Esculetin had antioxidant, anticancer, hepatoprotective properties and radical scavenging activity against oxidative stress [40]. Data revealed that utilization of *Cynara* extracts can be a complementary supplement beside the medical treatment of hyperglycemia by improving the antioxidant and anti-inflammatory status related to diabetes mellitus possibly due to the presence of several anti-inflammatory, antioxidant and hepato-protective phytochemicals belonging to phenolic, carotenoids and flavonoids groups.

CONCLUSION

The antioxidant capacity of *Cynara ScolymusL.* extracts decreased inflammation related to diabetes mellit us as deduced by rendering ESR level to normal values as the negative control, ascertaining its ability as a nti-inflammatory agent. Also, the leaf and pulp extracts contained esculetin and astaxanthin, respectively, owning hepato-protective properties that contributed in maintaining serum ALT in a safe level as the nega tive control. So, the main purpose of the present study was established and it is recommended to encourag e the consumption of *Cynara* leaf and pulp extracts on a wide range for decreasing oxidative damages and maintaining health.

REFERENCES

1. N. Khan, D. N. Syed, N. Ahmad, H. Mukhtar., 2013. Antioxid Redox Signal. 19(2): 151-162.
2. F.Pérez-García, T.Adzet, S. Cañigueral, 2000, Free Radical Research, 33(5), 661–665.
3. A. Pradhan., 2007. Nutr. Rev., 65: S152-S156.
4. R.P. Kalsait, P.B. Khedekar, A.N. Saoji, KP. Bhusari., 2011. Trop. J. Pharm. Res., 10(1): 41-45.
5. P.A. Low, 1997. Diabetes, 46: 38-41.
6. National Research Council (NRC), 1995. Nutrient Requirements of Laboratory Animals, 5th ed. National Academy Press, Washinngton, DC.
7. Seida
8. K. Ghanem, M. Ramadan, A.R. Farrag, H. Ghanem, A. Farouk, 2009. Pol. J. Food Nutr. Sci., 59: 175-181.
9. W., M. O'Leary (1989): Practical Handbook of Microbiology, page 634.
10. A.M. Liviu, M. Cristina, F. CHIRILA, D. S.DEZMIREAN, F.I.T Nicodim, 2010, Not. Bot. Hort. Agrobot. Cluj. 38(3): 40-44.P.
11. Prieto, M. Pineda, M. Aguilar., 1999. Analytical biochemistry 269(2): 337–41.

12. V. L. Singleton, R. Orthofer, R. M. Lamuela-Raventós., 1999. Methods in Enzymology, 299C:152-178.
13. J. Zhishen , T. Mengcheng, W. Jianming,1999. Food Chemistry, 64, 555-559.
14. M. Oyaizu, 1986 , Jap. J. Nutr. 44: 307-315.
15. J.B. Harborne, 1998, Phytochemical Methods - A Guide to Modern Techniques of Plant Analysis. Chapman and Hall, London. pp. 182-190.
16. V.S. Stoll, ,J.S. Blanchard, 1990, Buffers: Principles and Practice. Meth. Enzmol. 182, 24-38.
17. NRC 1995 Nutrient Requirements of Laboratory Animals, 5th ed. National Academy Press, Washinngton, DC.
18. W.T. Caraway, 1975. Standard methods of clinical chemistry. Seliyson, D. Ed., Academic Press, New York and London.
19. S. Reitman, S. Frankel, 1957. Am. J. Clin. Path., 28: 56.
20. R. Henry, 1964, Clinical Chemistry. Principles and Techniques, Harper-Row, New York, pp. 182.
21. B.T.Doumas , W.A. Watson ,H.C. Biggs, 1971, Clin. Chem. Acta. 31: 87.
22. Westergren A. ,1957, Triangle 3(1): 20-25.
23. M. M. Cowan, 1999, Clin. Microbiol. Rev. 12(4): 564-582
24. M. L. Brigden. 1999, Physician., 60: 1443-50.
25. A. N. Khan, R. A. Khan, M. Ahmad, N. Mushtaq. 2015, j. Pharmacognosy and Phytochemistry 2015; 3(6): 217-220.
26. D.X., Hou , M. Fukuda, J.A. Johnson, K. Miyamori, M. Ushikai, M. Fujii 2001, Int J Oncol. ;18:1175–1179.
27. H.S. Jang, S.H. Kook, Y.O. Son, et al., 2005, Biochim Biophys Acta. ;1726:309–316.
28. M.Funakoshi-Tago, K.Nakamura, K. Tago, T.Mashino, T. Kasahara, 2011, Int Immunopharmacol. ;11:1150–1159.
29. H. Palafox-Carlos, J. Gil-Chávez, R. R. Sotelo-Mundo, J. Namiesnik, S. Gorinstein , G. A. González-Aguilar., 2012, Molecules, 17: 12657-12664.
30. D. Angelino, L. Gennari, M. Blasa, , R. Selvaggini, S. Urbani, S. Esposto, , M.Servili, P. Ninfali, 2011. J. Agric. Food Chem. 59, 2011–2018.
31. M.A. Carluccio , M. Massano, M.A. Ancora, , E. Scoditti, C. Storelly, C. Giovannini, P. Matarrese, M. D'Archivio, R. Masella, 2007. Nutr. Metab. Cardiovasc. Dis. 17: 535-545.
32. M. Erden, L.Ediz, Ö. Hız, Y. Tuluce, H. Ozkol, M. Toprak, F. Demirdag, 2012, International Journal of Clinical Medicine, 3: 377-382.
33. M. Mourelle, Villalon C., Amezcua J.L., 1988, J. Hepatol., 6: 337-342.

34. B. Dashti, I. Jeppson, B. Hagerstrand, U. Hultberg, M. Srinivas, S. Abdulla, H. .Bengmark, 1989, Eur. Surg. Res. 21: 83-91.

35. M.Rojkind, 1992, Pharmacol. Ther. 53: 81-104.

36. V. Charlton-Menys, P.N. Durrington,, 2007 , Drugs., 67, 11-16.

37. J.P. Yuan, J. Peng, K .Yin, J.H. Wang, 2010, Mol. Nutr. Food Res.; 55(1): 150- 165.

38. E.Yamashita, 2013, Functional Foods in Health and Disease, 3(7): 254-258.

39. H. Uzkeser, E,Cadirci, Z. Halici, F.Odabasoglu, B. Polat, T.N.Yuksel, S. Ozaltin, F. Atalay, 2012. Mediators of Inflammation, Article ID 438912, 10 pages.

40. Y. Kimura, M. Sumiyoshi, 2015, Eur J Pharmacol 746: 115-125.

Corresponding author: Ghadir A. El-Chaghaby,

Regional Center for Food and Feed, Agricultural Research Center, Giza, Egypt

ghadiraly@yahoo.com

Online publication Date: 20.08.2018