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POLYPHENOLS COMPOUNDS INVESTIGATION OF LEAVES AND CALLUS ARTICHOKES (*CYNARA COLYMUS* L.)

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Abstract

Since ancient times humans have adopted herbs and wild plants in the treatment of diseases. Medicinal plants were used more at the beginning of the 20th century to treat many diseases through the extraction of active chemical compounds. It is, therefore, necessary to detect the active substances of medicinal plant extract. *Cynara scolymus* L. is a medicinal plant mentioned in British and European Pharmacopeia. Artichoke is considered as medicinal plant, due to its nutritional and phytochemical composition, especially high proportion of phenolics compound. The phenolics include phenolic acids such as (Cynarin, Chlorogenic acid, Luteolin Salicylic acid and caffeic acid). The present study included phytochemicals (secondary metabolites) Screening of the crude extracts (leaves, friable and compact callus) and the five major active phenolic compounds were identified from the crude methanolic extracts of leaves, friable and compact callus using high performance liquid chromatography (HPLC) analysis. Two of these compounds, Salicylic acid and 5-o-Caffeoylquinic acid (Chlorogenic) were found to be in high concentration in leaves extract that recorded 526.182 µg/ml and 5222.421µg/ml, respectively. The other three compounds, 1,3-di-o-Caffeoylquinic acid (Cynarin), Luteolin, and Caffeic acid were found to be in high concentration in friable callus extract that recorded 3205.153 µg/ml, 940.983 µg/ml, 380.437 µg/ml respectively.

Key words: *Cynara scolymus*, Callus, HPLC, Chlorogenic acid, Cynarin

Introduction

Artichokes (*Cynara scolymus* L.) AC *Asteraceae* family is a medicinal and edible plant found in the Mediterranean (Chakravarty, 1976).The immature head is the part that carries food and was used in ancient civilizations such as ancient Egyptian and Greek civilizations(Magied *et al.*, 2016). AC used medical purposes for its content of the polyphenolic constituent. The phenolics include cynarin (1,3-di-o- caffeoylquinic acid), Chlorogenic (5-o-Caffeoylquinic acid), and caffeic acid (Christaki *et al.*, 2012). Callus cultures generally are induced from any explant structure such as leaf, stem, and root by put explants on culture media containing a comparatively high level of auxin and low level of cytokinin. Callus initiation depends by type and concentration of growth regulators and nutrients vitamins in culture media. Furthermore, the response of explant on culture media also depends on the exposure to different lighting periods (Bekheet, 2011). Tissue culture is a great promise for controlled production of a plenty of useful secondary metabolites. In the search for alternatives to production of medicinal compounds from plants, biotechnological approaches, specifically plant tissue cultures, are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites (Filova, 2014). To obtain more comprehensive knowledge of the phenolic compounds of the medicinal plant (*Cynara scolymus* L.) and it compared between leaves and callus, the aim of this study were to a determination of phenolic compounds in leaves, friable and compact callus extracts by using HPLC.

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Materials and method

Plant material

Artichoke seeds were obtained from College of Pharmacy- University of Baghdad. During October 2016, the seeds were germinated in pots and grown in the green house; the plants were also grown in private field at Al-Nahrain University. The leaves were collected during March and April 2017.

Preparation of culture medium for callus induction

To prepare 1000ml MS medium, 34.1g of the ready made was dissolved powder in 800ml distilled water, adding 3% sucrose, and then adding growth regulators. The pH was adjasted (5.7-5.8) using (1N NaOH or 1N HCl); the volume was adjusted to 1000ml and eight g/1 of agar was added to stiffen the medieum. For callus induction, MS medium was supplmented with all the possible commbinatios for diffrent concentrations (0.0, 1.0, 3.0 or 5.0 mg/l) of naphthalin acetic acid (NAA), and diffrent concentration (0.0, 0.2, 0.5 or 1.0 mg/l) of benzyl adenin (BA). The medium components were kept on a hotplate with magnetic stirrer close to boiling. The culture media were autoclaved at 121°C, 1.04 kg/cm^2 pressure for 15 min; then 10 ml were poured into glass vial, and placed at room temperature to cool and be ready for using (Ullah *et al.*, 2007).

Explants sterilization

Leaf explants were before sterilized with 70% (v/v) ethanol surface for 10 seconds, must be washed with tap water. After rinsing three times with sterile DH_2O, the explants were dipped 1.0% V/V of sodium hypochlorite solution containing (6.25% Cl_2) at three periods 10 min for each concentration; then swill three times with sterile DH_2O. The sterilization was conducted inside the laminar air flow cabinet (Daud *et al.*, 2012).

Preparation of methanolic leaves and callus extract

The methanolic extract of *Cynara scolymus* was prepared by moistening 50 g of dried powdered samples in 250 ml of methanol 80% for 24 h; the extract was filtered by using filter paper. The filtrate was subjected to evaporation using a rotary evaporator in order to remove the solvent. The extract was used for phytochemicals detection (Karthishwaran *et al.*, 2010).

Phytochemicals Screening

Phytochemicals Screening of the crude extracts (leaves, friable and compact callus) were carried out according the following, Alkaloids test according to a method (Jha *et al.*, 2012), Flavonoids test (Hossain *et al.*, 2013), Tannins test, Anthraquinones test, Saponins test according to a method(Auwal *et al.*, 2014), Terpenoids test, Phenolic compounds test and Cardiac glycoside test according to a method (Kumar Bargah, 2015).

Fractionation and identification of phenolic compounds by HPLC

The polyphenolic compounds of artichoke extracts were fractionated and identified for phenolic compounds by HPLC, according to the method described by (Al-Farsi & Lee, 2008). The eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer. The main compounds were separated on FLC (Fast Liquid Chromatographic) column under the optimum conditions. Column: phenomenex C-18, 3μm using a hypersil C18 reversed- phase column with 5μm particle size (100 × 4.6 mm I.D) column. The mobile phase was linear gradient of solvent A (0.1% trifluoroacectic acid) in deionized water and solvent B (100% of acetonitrile). The program was started with elution with 95% A (0-1min); that a linear gradient was used until 10 min, lowering A to 20%; from 17 min to 24 min, and A decreased to 0%. The flow rate was 1.3 ml/min, and the runs were integrated at 280 nm for each Phenolic derivatives. Scanning was performed from 200 to 600 nm. Phenolic compounds were identified by comparing retention times and UV-VIS spectra with those of pure standards and the range of calibration curves. The repeatability of the quantitative analysis was ± 4%. The results were expressed as microgram/ml of each compound per total phenolic compounds. Phenolic compound of extract calculted according to the following equation: Concentration of sample (μg/ml)

$$= \frac{Area\ of\ sample}{Area\ of\ standard} \times \text{conc. of standard} \times \text{dilution}$$

Results and dscussion

Callus Induction Culture

For callus induction, leaves were used as explants to obtain callus form AC. The auxin, Naphithaleneacetic acid (NAA) and the cytokinin, Benzyaladenine (BA) were used in different combination. The data showed statistically significant differences among the means (table 1).

Callus induction culture was calculted according to the following equation:

$$\text{Callus induction culture \%} = \frac{No.of\ explant\ produced\ callus}{Total\ no.of\ explants\ cultured} \times 100\%$$ (Osman *et al.*, 2016).

The combination of NAA (5.0 mg/l) plus BA (2.0 mg/l) produced 100% response for callus induction that appeared as friable after 4 weeks in culture (fig- 1); while the combination of NAA (3.0 mg/1) and BA (1.0 mg/1) was produced 86.67% friable callus (fig- 2).

The combination of NAA (5.0 mg/1) and BA (0.5 mg/1) produced compact callus with brown and green spots after 4 weeks in culture (fig- 5); the callus induction was (83.33%) as compared to the control treatment that

Fig 1: Callus induction on *Cynara scolymus* from leaf explants grown on MS medium, It includes NAA (5mg/l) and BA (2mg/l) after four weeks of incubation at 23±1C°.

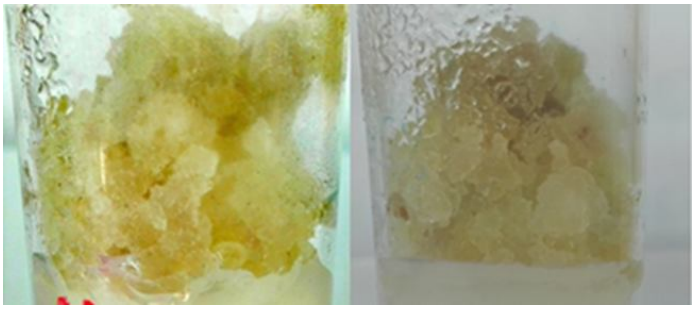

Fig 2: Callus induction on AC from leaf explants grown on MS medium, It includes NAA (3mg/1) and BA (1mg/1) after four weeks of incubation at 23±1C° .

gave 0.0 %.

Several researchers studied callus formation in AC. (Ordas *et al.*, 1990) mentioned successful callusing response from bracts globle artichoke using MS medium containing a comebination of 5 mg/l NAA and 2 mg/l BAP. (Brutti *et al.*, 2000)described a microproipagation protacol for AC *;* shoot apices were estublished on basic culture medium consesting of MS medium containing 3mg/l NAA and 4mg/l cyclodextrins. Leaf segments, taken from *invitro* grown shoots were subcultured on MSmedium contained 2 mg/l BA and 5 mg/l NAA according to (El-Bahr *et al.*, 2001); (Shawky & Aly,

Fig.3: Callus induction of AC from leaf explants grown on MS medium contining NAA, (5.0mg/1) and BA (0.5 mg/1) after 4 weeks of incubation at 23±1C°.

2007). In another study, a protocol for *in vitro* multiplication of AC using axillary buds was established using medeium contaning 1mg/1 kinetin and 0.1 mg/1 NAA after removal of apical buds, leaves and roots from seedlings (El Boullani *et al.*, 2012). Leaf explants were cultured on MS medium, for callus induction added with 5 mg/l NAA, 2 mg/l Kin, and 0.1 mg/l GA3 (El Bahr, 2014). The data in (table 1) indicated that callus was induced only when using different combinations of the auxin NAA and the cytokinins BA. When one growth regulators was used in the treatment, the explants produced no callus. Cell division appears to be reguluted to his work by the jont effect of auxins and cytoknins. Auxins seems an influence on DNA replacation, while cytokinins affected somecontrol over the proceedings leading to mitosis (Koens *et al.*, 1995). An auxin is in general was demand for the indaction of callus from explants. Cells, that for responding to auxins, come back to a dedifferenatiated state and begin to divide. (LoSchiavo *et al.*, 1989) get it that auxins cause DNA to shows more methyleated than usually and, he was proposed that this could be necessary for the reprogramiming of differenitiated cells. Cytokinins are adding in medium tissue culture seem to be indispensable that

Table 1: Effect of various concentrations of (NAA, BA) and their combinations on the response of callus induction from *Cynara secolymus.*

NAA (mg/l)	Concentration of BA (mg/l)								Mean (NAA)
	0.0		0.5		1.0		2.0		
	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%	Mean ± SD	%	
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	0.0	6.00±1.0^{c}	60.00	3.33±1.15^{c}	33.33	4.67±0.57^{c}	46.67	3.50±2.43^{c}
3.0	0.0	0.0	6.67±0.57^{b}	66.67	8.67±0.57^{a}	**86.67**	7.33±1.52^{b}	73.33	5.67±3.57^{b}
5.0	0.0	0.0	8.33±1.15^{a}	**83.33**	6.33±0.58^{b}	63.33	10.00±0.0^{a}	**100**	6.16±3.99^{a}
Mean (BA)	0.0	0.0	**5.25±3.36^{a}**	-	**4.58±3.44^{b}**	-	**5.50±3.91^{a}**	-	-

LSD (p=0.05)=0.617, R=10

for plant cell division of plant tissue. He have been proposed that cytoknins may be necessary to streamline the synthasis of proteins interested of the function and structure of the mitotic spinidle appartus. callus is produced when added cytoknin and auxin in the growth culture medium were available together medium (George *et al.*, 2007). auxins and cytokinins are adding together in tissue culture seem to be necessary for plant tissue culture, This explains why there is no callus induction when growth regulator alone is lost in a medium growth culture.

Phytochemical screening of methanolic crude extract

The phytochemical screening results for artichoke extract AC from leaf, friable and compact callus are presented in table 2. The extract contents are crude of Alkaloids, Flavonoids, Tannins, Saponins, Terpenoids, Phenolic compound, and Cardiac glycosides; while Anthraquinones was absence in the extract.

Phytochemical screening is in agreement with Oa *et al*. (2013)(Oancea *et al.*, 2013); Ben Salem *e* (2017) (Ben Salem *et al.*, 2017) revealed the pres of cardiac glycosides, flavonoid, tannin, saponin, terpe and alkaloids in *Cynara scolymus* leaves extra

Identification of phenolic compounds in lea friable callus, and compact callus of Artichoke

Table 2: Detection Phytochemical Compounds *Cy scolymus* extracts

Compact callus	Friable allus	Leaves	Phytochemical compounds
+	+	+	Alkaloids
+	+	+	Flavonoids
-	+	+	Tannins
+	+	+	Saponins
+	+	+	Terpenoids
+	+	+	Phenolic compou
+	+	+	Cardiac glycosid
-	-	-	Anthraquinones

The phenolic compounds of the leaves, friable and compact callus of artichoke extracts were identified by HPLC. The phenolic compounds include (Chlorogenic acid, Cynarin, Luteolin, Caffeic acid and Salicylic acid). Results of retention time and area (**µ**volt) are shown in table 3, and Fig- 4 that presented phenolic compounds standard.

$$\text{Concentration of sample } (\mu g/ml) = \frac{Area\ of\ sample}{Area\ of\ standard} \times \text{conc. of standard} \times \text{dilution factor}$$

(1) (Al-Farsi & Lee, 2008). Conc. of standard =50 µg/ml, dilution factor =50

The results shown in table 4 and (fig. 5, 6, 7) indicated that the phenolic compound [5-o-Caffeoylquinic acid (Chlorogenic acid)] had high concentration in leaves

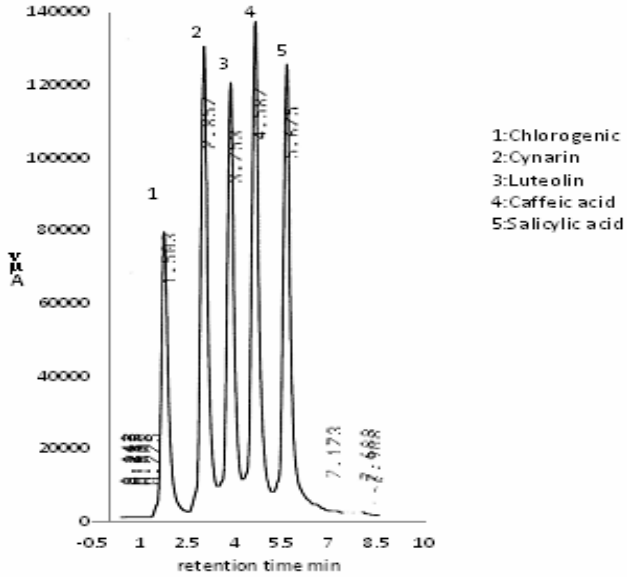

Fig. 4: Phenolic compounds Retention time (min), Area (µvolt) for the standard analysis by HPLC.

Table 3: Retention time (min) and Area (µvolt) of phenolic compounds for methanolic extract (standard, leaves, friable and compact callus) that identified by HPLC.

Phenolic compounds	Standard		Leaves		Friable Callus		Compact Callus	
	Retention time (min)	Area (µvolt)	Retention time (min)	Area (µvolt)	Retention time (min)	Area (µvolt)	Retention time (min)	Area (µvolt)
5-o-Caffeoylquinic acid (Chlorogenic)	1.503	68215	1.580	142499	1.550	73527	1.448	72356
1,3-di-o-Caffeoylquinic acid (Cynarin)	2.857	109540	2.917	127420	2.883	140437	2.800	121129
Luteolin	3.753	103259	3.822	25172	3.792	38866	3.832	23295
Caffeic acid	4.587	122497	4.675	17773	4.663	18641	4.558	14152
Salicylic acid	5.675	120291	5.825	25318	5.708	13280	5.625	6240

extract that recorded 5222.421µg/ml; while it was 2694.678 µg/ml for the friable callus, and 2561.762 µg/ml with compact callus. This is consistent with results of Moglia *et al*. (2011) (Moglia *et al.*, 2011) who indicated that chlorogenic acid accumulates preferentially in leaf tissue, compared with callus of *Cynara scolymus* when using HPLC analyses.

Table 4: Phenolic compounds (µg/ml) for extract of leaves, friable callus, and compact callus of *Cynara scolymus* that identified by HPLC

Phenolic acids	Leaves extract (µg/ml)	Friable Callus extract (µg/ml)	Compact Callus extract (µg/ml)
5-o-Caffeoylquinic acid (Chlorogenic)	5222.421	2694.678	2561.762
1,3-di-o-Caffeoylquinic acid (Cynarin)	2908.07	3205.153	2764.49
Luteolin	609.438	940.983	563.994
Caffeic acid	362.723	380.437	288.823
Salicylic acid	526.182	275.997	129.68

The phenolic compound [1, 3-di-o-Caffeoylquinic acid (Cynarin)] produced 2908.07 µg/ml in leaves extract, and 2764.49 µg/ml in compact callus. Cynarin showed the highest percentage with friable callus that recorded 3205.153 µg/ml. This result is compatible with Trajtemberg *et al*. (2006) (Trajtemberg *et al.*, 2006) who indicated that calluses gave a higher content of cynarine compar to *invivo* leaves of *Cynara cardunculus*. So, *in v* tissue cultures can be used as a source for obtai cynarine for pharmaceutical purposes.

Luteolin was found to be in high concentratio friable callus that recorded 940.983 µg/ml, comparing leaves that gave 609.438 µg/ml**.** Luteolin was in percentage with compact callus that recorded 563.99 ml. Colceru-Mihul *et.al.*,2016 (Colceru-Mihul *et al.*, 2 indicated that *Cynara scolymus* produced h concentration of polyphenols: luteolin, luteolin-7-gluco caffeic acid, and chlorogenic acid. Luteolin was also f in leaves and heads in two types of artichoke (Magie *al.*, 2016).

Caffeic acid was found to be in high concentratio friable callus that recorded 380.437µg/ml, and 362.723 µg/ml for leaves extract; while it was lo compact callus that recorded 288.823µg/m.

Salicylic acid showed the highest concentration leaves (526.182 µg/ml), but it was 275.997 µg/ml in fri callus. Salicylic acid produced the lowest concentration with compact callus (129.68 µg/ml). Phenolic compound (caffeic acid and salicylic acid) were also found in leaves and heads for two varieties of artichoke (Magied *et al.*, 2016).

Amounts of phenolic compounds noticed under environmental stress can be considered both a common response of plant adaptation to stress conditions, improvement evolutionary capacity, and a way of leading and storing carbon skeletons produced by photosynthesis

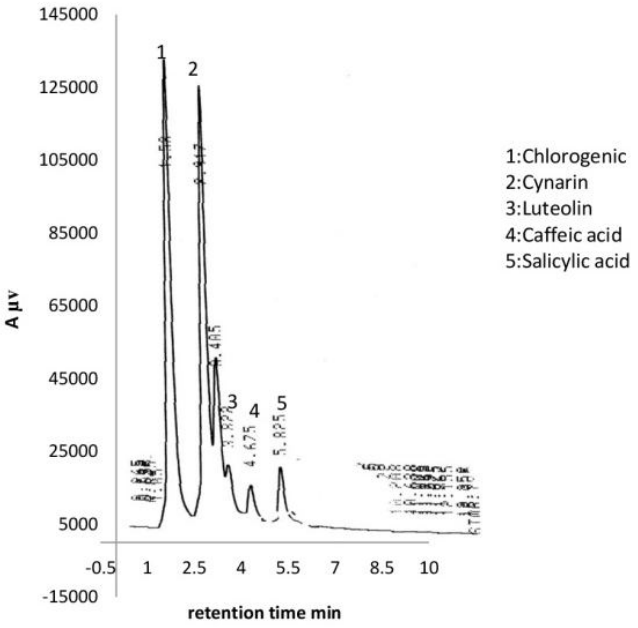

Fig. 5: Phenolic compounds retention time (min), area (µvolt) for the leaves extract analysis by HPLC.

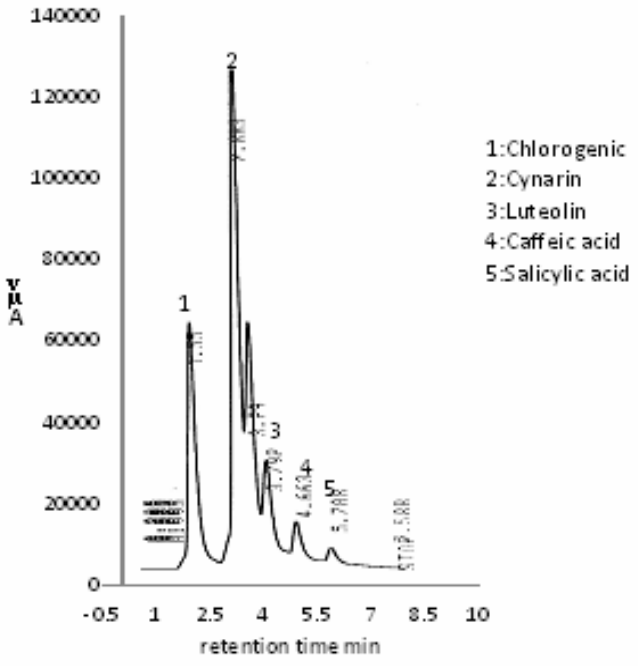

Fig.6: Phenolic compounds retention time (min), area (µvolt) for the friable callus analysis by HPLC.

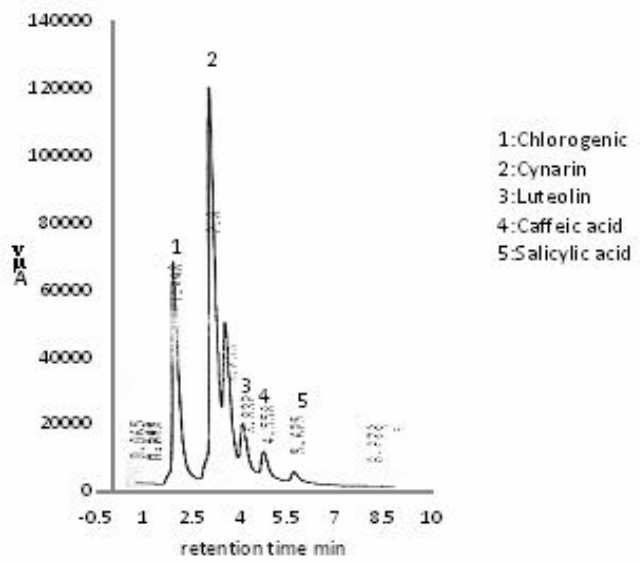

Fig 7: Phenolic compounds retention time (min), area (μvolt) for the compact callus analysis by HPLC

during periods when plant growth is curtailed. The motivation gene expression of phenolic metabolite pathways by biotic and abiotic stress is often acted by signaling molecules such as salicylic acid and jasmonic acid (Caretto *et al.*, 2015).

The HPLC analysis results indicated that concentrations of the phenolic compounds (Cynarin, Luteolin and Caffeic acid) was high in friable callus (*invitro* techniques) extract of *Cynara scolymus* , but chlorogenic acid and salicylic acid concentrations was high in leaves extract.

Higher plants synthesize an amazing diversity of phenolic compound. Phenolics are defined as secondary metabolites or natural products because, originally, they were considered not essential for plant growth and development (Caretto *et al.*, 2015). Phenolic compound is produced in the plant due to the interaction of plants with the environment. The type and quantity of phenols produced by the plant depends on the type of environmental stresses on the plants (Noel *et al.*, 2005). Castro *et al*. (2016) (Castro *et al.*, 2016) found that *in vitro* techniques both promote and increase the production of phenolic compounds in callus of *B. verbascifolia*. Possible increased production of *in vitro* secondary metabolism in medicinal plants is due to variation of tissue culture conditions, including alteration in types and concentrations of plant growth regulators (Palacio *et al.*, 2012). In plant tissues, that of cause increased amounts of phenolic compounds noticed under environmental stress can be considered both a common response of plant adaptation to stress conditions, improvement evolutionary capacity, and a way of leading and storing carbon skeletons produced by photosynthesis during periods when plant growth is curtailed. The motivation gene expression of phenolic metabolite pathways by biotic and abiotic stress is often acted by signaling molecules such as salicylic acid and jasmonic acid (Caretto *et al.*, 2015).

Conclusion

Callus cultures of Cynara scolymus can be induced on leaf explants and maintained on MS medium containing 5mg/l NAA and 2mg/l BA. Combination between NAA concentration 3.0 mg/l and BA concentration 1.0mg/l achieved response callus induction product friable callus too. Callus was induced only when using different combinations of the auxin NAA and the cytokinins BA, When one growth regulators were used in the treatment, the explants produced no callus.

Five major active phenolic compounds were identified from the crude methanolic extracts of leaves, friable and compact callus using high performance liquid chromatography (HPLC) analysis. Two of these compounds, Salicylic acid and 5-o-Caffeoylquinic acid (Chlorogenic) were found to be in a high concentration in leaves extract. The other three compounds, 1,3-di-o-Caffeoylquinic acid (Cynarin), Luteolin, and Caffeic acid were found to be in a high concentration in friable callus extract.

References

Al-Farsi, M.A. and C.Y. Lee (2008). Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*, **108**: 977-985.

Auwal, M.S., S. Saka, I.A. Mairiga, K.A. Sanda, A. Shuaibu & A. Ibrahim (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (*Thorn* mimosa). *Veterinary research forum: An International Quarterly Journal* (ed by), p. 95.

Bekheet, S. (2011) In vitro biomass production of liver-protective compounds from Globe artichoke (*Cynara scolymus* L.) and Milk thistle (*Silybum* marianum) plants. *Emirates Journal of Food and Agriculture*, **23**: 473.

Ben Salem, M., H. Affes, K. Athmouni, K. Ksouda, R. Dhouibi, Z. Sahnoun, S. Hammami & K.M. Zeghal (2017). Chemicals Compositions, Antioxidant and Anti-Inflammatory Activity of Cynara scolymus Leaves Extracts, and Analysis of Major Bioactive Polyphenols by HPLC. *Evidence-Based Complementary and Alternative Medicine.*

Brutti, C., N. Apostolo, S. Ferrarotti, B. Llorente & N. Krymkiewicz (2000). Micropropagation of *Cynara scolymus* L. employing cyclodextrins to promote rhizogenesis. *Scientia horticulturae*, **83**: 1-10.

Caretto, S., V. Linsalata, G. Colella, G. Mita & V. Lattanzio (2015). Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. *International*

Journal of Molecular Sciences, **16**: 26378-26394.

Castro, A.H.F., K.d.Q. Braga, F.M.d. Sousa, M.C. Coimbra & R.C.R. Chagas (2016). Callus induction and bioactive phenolic compounds production from *Byrsonima verbascifolia* (L.) DC.(Malpighiaceae). *Revista Ciência Agronômica*, **47**: 143-151.

Chakravarty, H.L. (1976). Plant wealth of Iraq (a dictionary of economic plants): vol. 1. *Baghdad: Ministry of Agriculture & Agrarian Reform xiv, 506p.-illus., col. illus..(Ara) Icones. Geog*, **2**

Christaki, E., E. Bonos & P. Florou-Paneri (2012). Nutritional and functional properties of Cynara crops (Globe Artichoke and Cardoon) and their potential application: A review. *International Journal of Applied Science and Technology*, **2.**

Colceru-Mihul, S., A. Nita, A. Grigore, C. Bubuanu, E. Dreghici, E. Vamanu & D. Rughinis (2016). Selective fractions with antioxidant activity from Romanian cultivated Cynara scolymus L. *Scientific Bulletin. Series F. Biotechnologies*, **20**: 347-351.

Daud, N.H., S. Jayaraman & R. Mohamed (2012). Methods Paper: An improved surface sterilization technique for introducing leaf, nodal and seed explants of Aquilaria malaccensis from field sources into tissue culture. *Asia Pac. J. Mol. Biol. Biotechnol*, **20**: 55-58.

El-Bahr, M., K.A. Okasha & S. Bekheet (2001). In vitro morphogenesis of globe artichoke (*Cynara scolymus* L.). *Arab Journal of Biotechnology*, **4**: 119-128.

El Bahr, M. (2014). Callus production of Globe Artichoke and Milk Thistle: *In vitro* hypolipidemic and antioxidant activities. *World Journal of Pharmaceutical Research*, **3**: 1-17.

El Boullani, R., A. Elmoslih, A., El Finti, A., El Mousadik, A. & Serghini, M.A. (2012) Improved in vitro micropropagation of artichoke (*Cynara cardunculus* var. *scolymus* L.). *European Journal of Scientific Research*, **80**: 430-436.

Filova, A. (2014). Production of secondary metabolities in plant tissue cultures. *Research Journal of Agricultural Science*, **46.**

George, E.F., M.A. Hall & G-J.De Klerk (2007). *Plant propagation by tissue culture:* Volume 1. *the background*. Springer Science & Business Media.

Hossain, M.A., K.A.S. AL-Raqmi, Z.H. AL-Mijizy, A.M. Weli & Q. Al-Riyami (2013). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian pacific journal of tropical biomedicine*, **3**, 705-710.

Jha, D.K., L. Panda, P. Lavanya, S. Ramaiah & A. Anbarasu, A. (2012). Detection and confirmation of alkaloids in leaves of Justicia adhatoda and bioinformatics approach to elicit its anti-tuberculosis activity. *Applied biochemistry and biotechnology*, **168**: 980-990.

Karthishwaran, K., S. Mirunalini, G. Dhamodharan, M. Krishnaveni & V. Arulmozhi (2010). Phytochemical investigation of methanolic extract of the leaves of Pergularia daemia. *J. Biol. Sci.*, **10**: 242-246.

Koens, K., F. Nicoloso, T.B. Van Vliet, M. Harteveld, C. Boot, F. Van Iren, P. Mulder, K. Libbenga & J. Kijne (1995). Kinetics of 2, 4-dichlorophenoxyacetic acid content in an auxin-dependent suspension culture of Nicotiana tabacum cells. *Journal of plant physiology*, **147**: 383-390.

kumar Bargah, R. (2015). Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of Moringa pterygosperma Gaertn. *Journal of Pharmacognosy and Phytochemistry*, **4**.

LoSchiavo, F., L. Pitto, G. Giuliano, G. Torti, V. Nuti-Ronchi, D. Marazziti, R. Vergara, S. Orselli & M.Terzi (1989). DNA methylation of embryogenic carrot cell cultures and its variations as caused by mutation, differentiation, hormones and hypomethylating drugs. *Theoretical and Applied Genetics*, **77**: 325-331.

Magied, M.M.A., S.E.D. Hussien, S.M. Zaki & R.M.E. Said (2016). Artichoke (*Cynara scolymus* L.) leaves and heads extracts as hypoglycemic and hypocholesterolemic in rats. *Journal of Food and Nutrition Research*, **4**: 60-68.

Moglia, A., B. Menin, C. Comino, S. Lanteri & J. Beekwilder (2011). Globe artichoke callus as an alternative system for the production of dicaffeoylquinic acids. *VII International Symposium on In Vitro Culture and Horticultural Breeding 961* (ed by), pp. 261-265.

Noel, J.P., M.B. Austin & E.K. Bomati (2005). Structure–function relationships in plant phenylpropanoid biosynthesis. *Current opinion in plant biology*, **8**: 249-253.

Oancea, A., G. Roatã, S. Popescu, L. Pãun, I. Mateescu, A. Toma, A. Gaspar & M. Sidorof (2013). Phytochemical screening of the bioactive compounds in the most widespread medicinal plants from calarasi-silistra cross-border area. *Bulletin of the Transilvania University of Bra°ov*, 135-142.

Ordas, R.J., R. Tavazza & G, Ancora (1990). *In vitro* morphogenesis in the globe artichoke (*Cynara scolymus* L.). *Plant Science*, **71**: 233-237.

Osman, N.I., N.J. Sidik & A. Awal (2016). Effects of variations in culture media and hormonal treatments upon callus induction potential in endosperm explant of *Barringtonia racemosa* L. *Asian Pacific Journal of Tropical Biomedicine*, **6**: 143-147.

Palacio, L., J.J. Cantero, R.M. Cusidó & M.E. Goleniowski (2012). Phenolic compound production in relation to differentiation in cell and tissue cultures of *Larrea divaricata* (Cav.). *Plant Science*, **193**: 1-7.

Shawky, B. & U. Aly (2007). In vitro conservation of globe artichoke (*Cynara scolymus* L.) germplasm. *International Journal of Agriculture and Biology (Pakistan)*,

Trajtemberg, S.P., N.M. Apóstolo & G. Fernández (2006). Calluses of Cynara cardunculus var. cardunculus cardoon (Asteraceae): determination of cynarine and chlorogenic acid by automated high-performance capillary electrophoresis. *In Vitro Cellular and Developmental Biology-Plant*, **42**: 534-537.

Ullah, H., I. Ullah, S.A. Jadoon & H. Rashid (2007). Tissue culture techniques for callus induction in rice. *Sarhad Journal of Agriculture*, **23**: 81.