sciendo

ASN, Vol 5, No 2, Pages 23 – 31, 2018

Acta Scientifica Naturalis

Former Annual of Konstantin Preslavsky University – Chemistry, Physics, Biology, Geography

Journal homepage: http://www.shu.bg

Received: 09.2018

Accepted: 11.2018

Anti-proliferative activity of ethyl acetate extracts of *Tamarix gallica L.* grown at different climatic conditions in Algeria

Ouroud Fellah*[1], Samir Hameurlaine[2], Noureddine Gherraf[2], Amar Zellagui[3], Tahar Ali[1], Abdennabi Abidi[4], Muhammed Altun[5], Ibrahim Demirtas[5], Ayse SahinYaglioglu[5]

[1]Faculty of sciences, department of natural and life sciences, university of Badji Mokhtar Annaba, 23000, Algeria:

[2]Laboratory of Natural Resources and Management of Sensitive Environments, Larbi ben M'hidiniversity, Oum El Bouaghi, Algeria,

[3] Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department, Faculty of Exact Science and Life Science and Nature , University of Larbi Ben MhidiOum El Bouaghi, Algeria.

[4]Department of Process Engineering, Badji Mokhtar University, Annaba 23000, Algeria,

[5] Laboratory of Plant Research, Department of Chemistry, Faculty of Science, Uluyazi Campus, CankiriKaratekin University, Cankiri, Department of Chemistry Turkey

Abstract:

The aerial parts of T. gallica collected from three different locations (arid, humid and semi-arid) were extracted using ethyl acetate. The crude extracts were subjected to phenolic appraisal and antiproliferative activity using ELISA and xCELLigence assays. The total phenolic and flavonoids were evaluated using appropriate techniques to give a yield of total phenolics ranging between 238.46 and 348.56 mg GAE (Gallic acid equivalent)/g dry weight extract. The flavonoids yield was found to vary from 36.6 to 103.14 mg QE (quercetin equivalent)/g dry weight extract. Moreover, the extracts were tested against rat brain

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

tumor (C6) and human cervix carcinoma (HeLa) cell lines and displayed important differences in activity. These disparities highlighted the effect of climatic factors as quality determinants of secondary metabolites and therefore as a key control of the biological therapeutic effect.

Keywords: **Tamarix gallica; Geographic Origins; ethyl acetate; phenolic; flavonoids; anti-cancer activity.**

1. Introduction

The employment of plant extracts as natural healing agents has a long term history through civilizations. Many traditional herbs are well known for healing of simple diseases to serious and dangerous ailments including different types of cancer, owing to their efficacy as potential sources of natural antioxidants [1,2]

Recently, various herbal extracts have fascinated many researchers' reflection for cancer treatments. A number of medicinal plants and their bioactive compounds have shown anticarcinogenic and antiproliferative effects on cancer cells. Currently, over 60% anti-cancer agents are derived from natural sources, including plants, marine organisms, and micro-organisms. A variety of reports have shown the capability of phytochemicals to offer protection against free radical induced disorders due mainly to their content of flavonoids and phenolics in free or complex forms. These compounds have been identified and quantified in several fruits and vegetables and showed a high correlation with antioxidant activity [3,4]

The genus *Tamarix* (Tamaricaceae) is represented in Algeria by ten species. *Tamarixgallica* was reported to be useful in leucoderma, spleen trouble and eye diseases. Antimicrobial and antioxidantactivity of *Tamarixgallica* have also been reported. It can be used as prophylactic and therapeutic remedies to cure malaria as folk medicine. Moreover, it is reported to have anticancer effect on human colon cancer cells involving Erk1/2 and p38 action on G_2/M cell cycle arrest [5-9].

In our study, we have carried out the antiproliferative activity of ethyl acetate extracts of the plants *Tamarix gallica* collected from three different geographical origins (arid, semi-arid and humid) so as to highlight the impact of environmental conditions on the content of secondary metabolites and consequently on the remedial potency of such species.

2. Materials and Methods

2.1. Plant material

The aerial parts of *T. gallica* collected from Oum El Bouaghi (site 1: semi-arid), El Taref (site 2: humid) and Ouargla (site 3: arid). The plant was identified by Pr. A. Zellagui, Oum El Bouaghi University, Algeria. Voucher specimens (TG1, TG2, and TG3 for *T. gallica* of the 3 sites respectively) were deposited in the Laboratory of Natural Resources and Management of Sensitive Environments, University of Oum El Bouaghi, Algeria

2.2. Extraction

2.2.1. Ethyl acetate extract:

100 g of dry plant material of each sample was subjected to overnight extraction using ethyl acetate. After separation, the organic phase was evaporated and the crude extract was weighed and prepared for further analysis.

EA-semi arid: ethyl acetate extract of *T. gallica* from Oum El Bouaghi (site 1)

EA-humid: ethyl acetate extract of *T. gallica* from El Tarf (site 2)

EA-arid: ethyl acetate extract of *T. gallica* from Ouargla (site 3)

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

2.3. Total phenolic content :TPC

The Total phenolic content of each extract was determined using the folin-ciocalteau reagent method according to the method of Singleton [10].0.5 mL of the diluted solution of extracts in methanol was added to 2.5 mL of FCR (diluted 1/10 with distilled water) and mixed. After 5 min, 2 mL of sodium carbonate water solution Na_2CO_3(75g/L) was added to the mixture and incubated at 40 °C for 30 min. Results were expressed as mg of Gallic acid equivalent (GAE)/g of dry extracts. Gallic Acid was used as a standard. All samples were analyzed in triplicates.

2.4. Total flavonoids content:TFC

Total flavonoids content of the plant extracts was estimated according to the aluminum chloride colorimetric method [11]. This method based on the formation of a flavonoid-aluminum complex having the maximum absorbance at 430 nm. 1 mL of each extract was mixed with 1 mL of 2% $AlCl_3$methanolic solution and then the absorbance was determined at 430 nm using UV-VIS spectrophotometer. Total flavonoids content was expressed as mg quercetin equivalent/g of dry extracts and determined from the regression curve whose equation is: $y = 0.0299\ X + 0.0979$, $R^2 = 0.9746$. All samples were analyzed in triplicates.

2.5. in vitro anti-proliferative activity

Anti-proliferative activity of extracts was evaluated in vitro by the evaluation of the inhibitory effect of phenolic on the growth of cells on C6 (rat brain tumor) and Hela cell lines using proliferation BrdU ELISA and xCELLigence assays [12].

2.5.1. Cell culture

The cells were grown in Dulbecco's modified eagle's medium (DMEM, Sigma), supplemented with 10% (v/v) fetal bovine serum (Sigma, Germany) and PenStrep solution (Sigma, Germany) at 37°C in a 5% CO_2 humidified atmosphere.

2.5.2. Cell proliferation assays

- ELISA assay:

The cells were plated in 96-well culture plates (COSTAR, Corning, USA) at a density of 3×10^4 cells per well. The activities of samples were investigated on 250, 100 and 50 µg/mL. The cells were then incubated overnight before applying the BrdU Cell Proliferation ELISA assay reagent (Roche, Germany) according to the manufacturer's procedure. The amount of cell proliferation was determined at 450 nm by using a microplate reader (Awareness Chromate, USA). Results were reported as percentage of the inhibition of cell proliferation, where the optical density measured from vehicle-treated cells was considered to be 100% of proliferation. The stock solution of the extracts were prepared in dimethyl sulfoxide (DMSO) and diluted with DMEM. DMSO final concentration is below 0.1% in all tests. 5-FU was used as standard compounds. Percentage of inhibition of cell proliferation was calculated as follows: $(1 - (A_{treatments} / A_{vehicle\ control})) \times 100$.

- xCELLigenceassay

The xCELLigence system was used with the disposable E-plate 96 for the measurements of extracts, controls and medium. The measurements base on the impedance difference was caused by the cells attached to the E-Plate 96.

The medium (100 µL) was added to each well of E-Plate 96 and placed into the incubator. E-plate 96 was inserted to the xCELLigence station, and the background impedance was measured during 1 min. Then,

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

50 µL of each cell suspension was placed in medium containing wells and adjusted to 2×10^4 HeLa cells mL^{-1}. The plate was held in a sterile cabinet at room temperature for 30 min for attaching the cells to the E-Plate 96 wells. Finally, HeLa cells were monitored every 10 min for adhesion, growth and proliferation in a period of up to 3 h *via* the incorporated sensor electrode arrays of the E-Plate 96. The extracts were added to wells of plate and adjusted the crude ethyl acetate extract concentrations of 250, 100, 50, and 10 µg/mL. The plates were then immediately placed in the incubator and monitored every 10 min during 48 h. The tests were replicated and repeated three times given with standard deviation bars [13].

2.6. Statistical analysis

The results of investigation in vitro of anticancer activity are means ± SD of six measurements. Differences between groups were tested with ANOVA. p values of <0.01 were considered as significant and analyzed by SPSS (version 11.5 for Windows 2000, SPSS Inc.).

The result of scavenging activity and total phenolic compounds were performed from the averages of samples reading mean ±SD (standard deviation) used excel 2003. All analyses were carried out in triplicates.

3. Results and discussion

3.1. Total phenolic contents

Phenolic compounds are bioactive components of plants and show health promoting activities. The properties of the extracting solvents significantly affected the yields, total phenolic and oxidant activity. As assessed by the folin-ciocalteu's method. The total phenolic contents in the extracts are presented as mg GAE /g DW of extracts as displayed in table 1.

Table 1: Total phenolics in the ethylacetate extracts of *T. Gallica* (mg GAE/g DW)

Site	**Total phenolics**
Semi-arid	238.46±0.16
humid	348.56±0.11
arid	296.16±0.14

The results exposed important variations in total phenolic amount from one site to another. Site 2 (humid) exhibits important phenolic content: 348.56 mg GAE/g DW. This is certainly due to environmental factors especially water and nutrients favorable for the biosynthesis of such elements.

3.2. Flavonoid content

The flavonoids content, expressed in milligram of quercetin equivalent per gram of dry weight extract (mg QE / g DW) is shown in table 2.

Table 2: Total flavonoids in the ethylacetate extracts of *T. Gallica* (mgQE/g DW)

sample	**flavonoid content**
EA-semi arid	62.18±0.01
EA-humid	36.6±0.10
EA-arid	103.14±0.06

Other studies reported important fluctuarions in phenolic and flavonoidcontents in *T. Gallica*. For instance, the methanolic extracts of *Tamarix gallica* in Southern Algeria were assessed for their phenolic and flavonoidcontents to yield 334.19 mg GAE/g of phenolics and 395.62 mgQE/g of flavonoids [14]. Another

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

study conducted on the methanolic extract of the aerial partsof *Tamarix gallica* collected in New Delhi, India showed total phenolic and flavonoid of 6.99498 mg/100g and 47.61905 mg/100g respectively [15]. The results revealed the significant correlations between climatic conditions and secondary metabolites.

3.3. Anti-proliferative activities

3.3.1. C6 cells

The antiproliferative activities of EA-semi arid, EA-humid, EA-arid, and 5-FU were determined against C6 cells. The antiproliferative activities of all the extracts showed an increase in activities with dose increase against C6 cells. EA-humid, EA-arid, were found to have antiproliferative activities greater than those of 5-FU against C6 cells at concentrations of 50 and 100 µg/mL (fig. 1). However, EA-semi arid has better antiproliferative activity compared to the standard compound at a concentration of 50 µg / mL. The potency of the inhibitions (at 100 µg / mL) against C6 cells was: EA-arid ~ EA-humid > 5-FU> EA-semi arid.

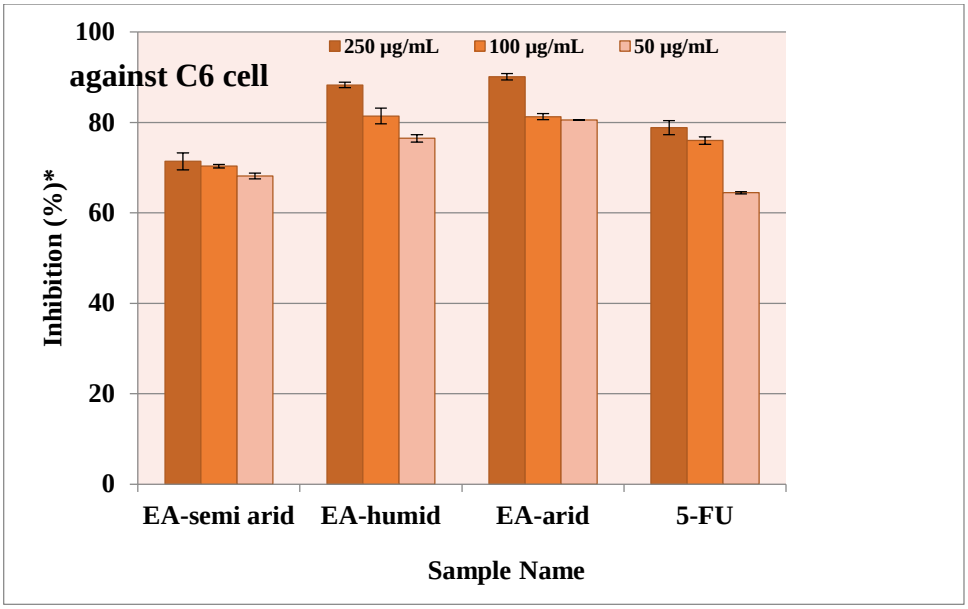

Figure 1. Antiproliferative activity of ethylacetate extracts against C6 cells.

*Each extract was tested twice in triplicate against cell lines. The data show the average of two individual experiments ($p < 0.01$)

3.3.2. Hella cells

The antiproliferative effect of the extracts was studied on HeLa cell line at the concentrations of 50, 100 and 250 µg/mL by using realtime cell analyzer xCELLigence technique. The system

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

quantifies the impedance variation in order to establish the Cell Index (CI) values at time points whose periods can be set by the operator. These impedance variations and thus the CI values depend on the cell activity at the base of the wells [16]. CI is a dimensionless parameter resulting as a relative change in measured electrical impedance to represent cell status. If it is decreasing, it shows us that the cancer cells are dying.

- Our records showed that the extracts displayed antiproliferative effect against HeLa cell line mainly at 250 µg/mL (Figure 2).

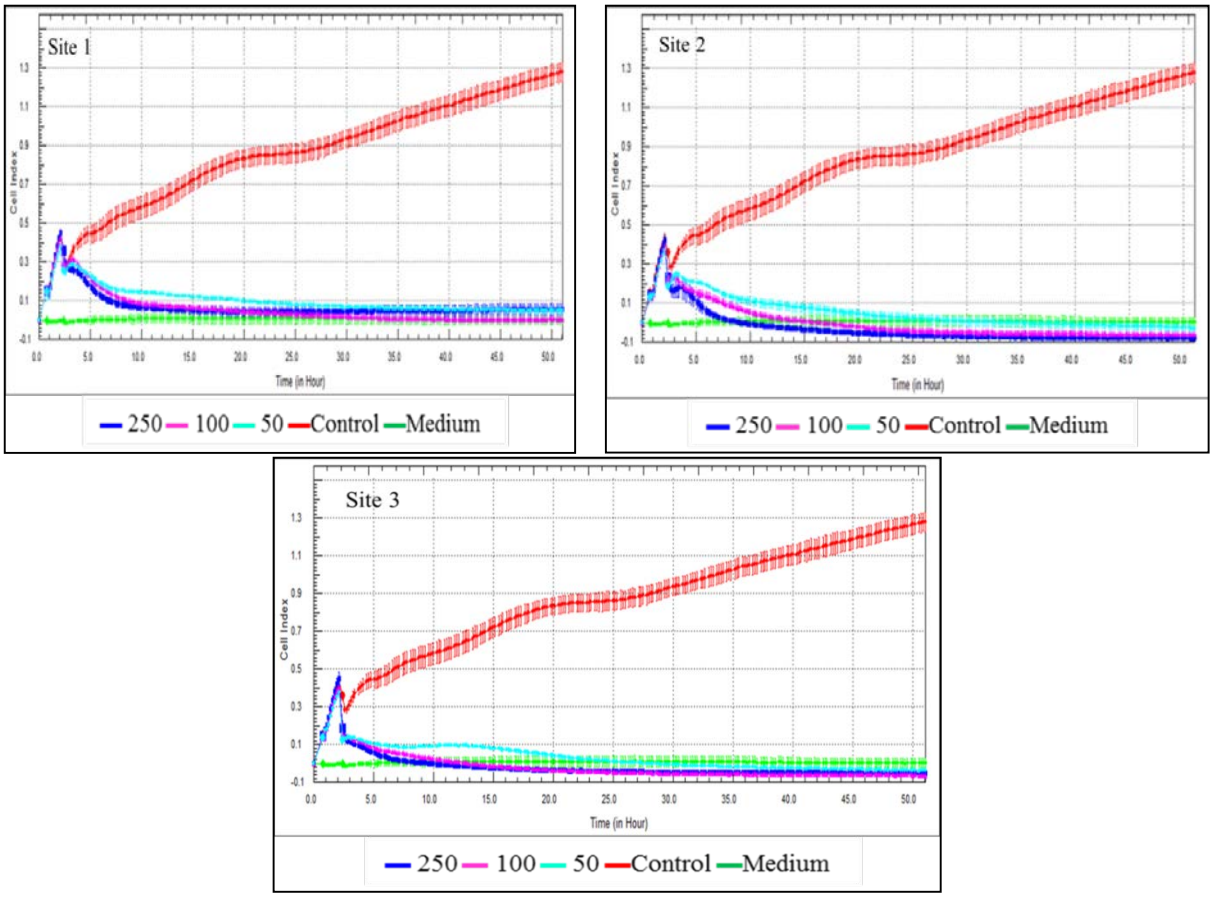

Figure 2. Antiproliferative activity of the ethylacetate extracts of *T. gallica*

For the ethyl acetate extract of *T. gallica*, good antiproliferative effects were observed at different concentrations. It seems that sites 2 and 3 extracts display a strong activity by decreasing the number of cancer cells. Previous reports stated the anticancer effect of *T. gallica* on human colon cancer cells and the possible primary mechanisms and showed that at 50 and 100 µg/mL, shoot, leaf, and flower extracts inhibited considerably Caco-2 cell growth [9]. Literature dealing with antiproliferative effect of *t. gallica* is relatively poor. Nevertheless, many studies reported anti-inflammatory, analgesic antioxidant, anti-hyperlipidemic, antiviral, cytotoxic, anti-Alzheimer and Type 2 Diabetes Diseases activities [14-20]

5. Conclusion

The amount of total phenolic compounds investigated in plant extracts is well correlated with the antiproliferative activity. It was revealed that the antiproliferative activity of ethyl acetate extracts of the *T.*

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

gallica is significant at 250 µg/mL when evaluated by BrdU ELISA and xCELLigence. The geographical locations and hence abiotic factors seem to have noteworthy role in affecting either the polyphenolic contents or the cytotoxic potency. Based on these results the plant can be a potential source of natural cytotoxic agents and *in vivo* studies are desired to extra substantiate the beneficial value of these extracts.

6. Conflict of Interest: The authors declare no conflict of interest

7. Acknowledgement: The authors would like to deeply thank Pr. Ibrahim Demirtas and his research team for his warm welcome and research facilities during our stay in the Laboratory of Plant Research, Department of Chemistry, Faculty of Science, Gaziosmanpaşa University, Uluyazi Campus, CankiriKaratekin University, Cankiri, Turkey.

References

[1]. Doudach, L.; Benbacer, L.; Meddah, B.; Faouzi, M.E.A.; Hammani, K.; El Mzibri, M.; Herrah, Y., Extract of 1:5 mixture of five Moroccan medicinal plants has cytotoxic effect on some human cancer cell lines, *Int. J. Pharm*. **2013**, *3*, 301-307.

[2]. Fattahi, S.; Ardekani, A.M.; Zabihi, E.; Abedian, Z.; Mostafazadeh, A.; Pourbagher, R.; Akhavan-Niaki, H., Antioxidant and apoptotic effects of an aqueous extract of *Urticadioica*on the MCF-7 human breast cancer cell line, *Asian Pac. J. Cancer Prev*. **2013**, *14*, 5317-5323

[3]. Ziech, D.; Anestopoulos, I.; Hanafi, R.; Voulgaridou, G.P.; Franco, R.; Georgakilas, A.G.; Pappa, A.; Panayiotidis, M.I., Pleiotrophic effects of natural products in ROS-induced carcinogenesis: the role of plant derived natural products in oral cancer chemoprevention, *Cancer Lett*. **2012**, *327*, 16–25.

[4]. Carmona-Ramirez, I.; Santamaria, A.; Tobon-Velasco, J.C.; Orozco-Ibarra, M.; Gonzalez-Herrera, I.G.; Pedraza-Chaverri, J.; Maldonado, P.D., Curcumin restores Nrf2 levels and prevents quinolinic acid-induced neurotoxicity, *J. Nutr.Biochem*. **2013,** *24*, 14–24.

[5]. Lefahal, M.; Benahmed, M.; Louaar, S.; Zallagui, A.; Duddeck, D.; Medjroubi, K.; Akkal, A., Antimicrobial Activity of Tamarix gallica L. Extracts and Isolated Flavonoids, *Advances in Natural and Applied Sciences*, **2010**, *4(3)*, 289-292

[6]. Lefahal, M.; Zaabat, N.; Djarri, L.; Benahmed, M.; M, Kamel.; Laouer, H.; Akkal,S., Evaluation of the antioxidant activity of extracts and flavonoids obtained from *Buniumalpinum* Waldst. & Kit. (Apiaceae) and *Tamarix gallica*L. (Tamaricaceae)Curr, *Issues Pharm. Med. Sci*., **2017,** *30(1)*, 5-8.

[7]. Kendour, Z.; Ladjel, S.; Gherraf, N.; Ouahrani, M.R., Antimicrobial activity of nine medicinal plants growing in the south of Algeria, *Annals of Biological Research*, **2010**, *1 (4):* 145-14.

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

[8]. Tagarelli, G.; Tagarelli. A.; Piro, A., Folk medicine used to heal malaria in Calabria (southern Italy), *J. Ethnobiol. Ethnomed*., **2010**, *6*, 27-27.

[9]. Boulaaba, M.; Tsolmon, Soninkhishig.; Ksouri, R.; Han, J.; Kawada, K.; Smaoui A.; Abdelly, C.; Isoda, H., Anticancer effect of *Tamarixgallica* extracts on human colon cancer cells involves Erk1/2 and p38 action on G_2/M cell cycle arrest, *Cytotechnology*. **2013**, *65(6),*927–936.

[10]. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteureagent, *Methods Enzymol*. **1999,** *299*, 152-178.

[11]. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stoker, P.; Vidal, N., Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds, *Food Chem.***2006**, *79*, 654-660.

[12]. Demirtas, I.; Sahin-Yaglioglu, A., Bioactive Volatile Content of the Stem and Root of *Centaureacarduiformis* C. subsp. *carduiformis* var. *carduiformis, Journal of Chemistry*. **2012**; doi: 10.1155/2013/125286.

[13]. Koldaş, S.; Demirtas, I.; Ozen, T.; Demirci, M.A.; Behçet, L., Phytochemical screening, anticancer and antioxidant activities of *Origanum vulgare* L. ssp. viride (Boiss.) Hayek, a plant of traditional usage, *J. Sci. Food Agric*. **2015**, *95*, 786-1482.

[14]. Tabet, A.; Boukhari; Abbes., Antioxidant and Antibacterial Activities of Two Algerian Halophytes, *Int. J. Pharm. Sci. Rev. Res.*, **2018**, *50(1),* 114-121

[15]. Chaturvedi, S.; Drabu, S.; Sharma, M., Antioxidant activity total phenolic and flavonoid content of aerial parts of *Tamarix gallica*, *International Journal of Phytomedicine*, **2012**, *4*, 174-180

[16]. Isik, S.; Karagoz, A.; Karaman, S.; Nergiz, C., Proliferative and apoptotic effects of olive extracts on cell lines and healthy human cells, *Food Chem*., **2012**, *134*, 29-36.

[17]. Al-Jaber, N.; Allehaib, L., The Pharmacological Activity of Some Tamaricaceae Plants, *Nat Prod Chem Res* **2017,** *5*, 262.

[18]. Syed, A. N.; Malladi, S.R.; CH. Pradeep, K.; Suhasini, B.; Sudheer, K.D., Anti-hyperlipidemicactivity of tamarix gallica extracts in triton x-100 induced hyperlipidemic rats, *International Journal Of Pharmacy & Technology*, **2015** , *6(4),* 7880-7895

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017

[19] Ionescu, D.; Aldea, I. M.; Bleotu, C.; Conomescu, M. C.; Matei, L.; Rizea, G. D.; Crisan, I.; Diaconu C. C.; Mihele, D., Evaluation of antiviral and cytotoxic activities of tamarix gallica and silybum marianum extracts. *FARMACIA*, **2014**, *62(2)*, 329-341

[20] Ben Hmidene, A.; Hanaki, M.; Murakami, K.; Irie, K.; Isoda, H.; Shigemori, H., Inhibitory Activities of Antioxidant Flavonoids from Tamarix gallica on Amyloid Aggregation Related to Alzheimer's and Type 2 Diabetes Diseases, *Biol Pharm Bull.* **2017**, 40(2), 238-241 *doi: 10.1248/bpb.b16-00801*.

Corresponding author:ouroud.fellah@yahoo.com

DOI: 10.2478/asn-2018-0017