Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2015; 7(6); 1231-1235

ISSN: 0975-4873

Research Article

Cytotoxic activity of flavonoid extracts from *Lepidium sativum* (Brassicaceae) seeds and leaves

Ait-Yahia O[1*], Bouzroura S A[2], Belkebir A[1], Kaci S[3], Aouichat A B[3]

[1]*Laboratory of vegetale physiology Faculty of biological Sciences, Houari Boumediene, University of Sciences and Technology, BP 31, El-Alia, Bab-Ezzouar 16111, Algiers, Algeria*
[2]*Laboratory of Applied Organic Chemistry, Houari Boumediene University of Sciences and Technology, BP 31, El-Alia, Bab-Ezzouar, 16111, Algiers, Algeria*
[3]*Laboratory of Cellular and Molecular physiopathogy Faculty of biological Sciences, Houari Boumediene, University of Sciences and Technology, BP 31, El-Alia, Bab-Ezzouar 16111, Algiers, Algeria*

Available Online:21st November, 2015

ABSTRACT
Lepidium sativum known as Garden cress belongs to Brassicaceae family, has been known centuries ago, this plant is widely distributed in Algeria, *Lepidium sativum* seeds is used in Algeria in folk medicine. In Europe the leaves of gardencress are consumed in salad. In this study three flavonoid extracts were obtained from *Lepidium Sativum* seeds and leaves: O-glycosides, C-glycosides and flavones/flavonols. The O-glycosides and C-glycosides were obtained by separation with ethyl acetate and butanol respectively. Whereas the Aglycones (flavones/ flavonols) were generated by acid hydrolysis. antitumoral activity has been tested towards HEp2 cells (Human Laryngeal Carcinoma Cells). At 57µg/mL the highest cytotoxic activity of the acetate ethyl extract (extract rich on O-glycosides) was observed against HEp2 cells, cells proliferation were reduced from 87% this effect is probably due to the apoptosis phenomena. All extracts obtained from *L. sativum* leaves exhibited a strong inhibition of cell proliferation but it has not provoked apoptosis.

Keywords: *Lepidium sativum*; Seed; Leaves; Apoptosis; Antitumoral; Flavones/flavonols; O-glycosides; C-glycosides

INTRODUCTION

Recent advances in plant sciences have led a great interest in the production of plant secondary metabolites for their medicinal and aromatic uses. It is revealed that the Brassicaceae is a high interesting family, present vegetables, plants rich on oil and the model spices of plant science. Among these plants *Lepidium sativum* L. plant and seeds are well known in the community of Arabic countries and some other of western Asia, although it is now cultivated in the entire world. L. *Sativum* seeds have been used in Algeria in different treatments. The seeds paste is applied to rheumatic joints to relieve pain and swelling. The seeds are chewed to treat sore throats, coughs, asthma and headaches. Seeds pounded in water are used to treat hiccoughs and stomach-aches. The seed oil is used as an illuminant and in soap manufacture. The seeds are used, fresh, dried or boiled consumed in drinks, either ground in honey or as an infusion in hot milk.. Its young leaves are eaten raw or cooked *Lepidium sativum* possesses several pharmacologique activities, leaves and seeds extracts were found to have Antihypertensive activity[1], seed extracts has proved hepato protective, hypoglycemic and used in treating bronchial asthma[2]. Chemical study has shown that seeds and leaves contain vitamin A, thiamine, riboflavin, niacin and ascorbic acid and secondary metabolites as sinapic acid and its choline ester (sinapin) and flavonoids. In addition we can also found in leaves sinapoylglucose, esters of caffeic, pcoumaric, ferulic, quinic acids and the esters of flavonoids[3,4]. Flavonoids (C6-C3-C6) can be classified in different subclasses (flavones, flavanones, flavonols, isoflavones, flavanols, chalcones and anthocyanins). It is revealed that many of these compounds are glycosylated under O-glycosides or C-glycosides forms[5,6].

The flavonoids and their glycosides have received a considerable interest because of their protective role against cancer and heart disease attributed of their antioxidant activity against reactive oxygen species. To our knowledge, the relationship between Aglycones (flavones/ flavonols) and their analogues O-glycosides and C-glycosides are not yet established for *Lepidium sativum* seeds and leaves. Our work has focused on the effects of various seeds and leaves extracts, (ether ethylique, acetate ethyl and butanolic fractions) on cancer cell proliferation and tumor growth of Human Laryngeal Carcinoma (HEp2) cells.

MATERIALS AND METHODS

Seeds collection

Seeds of *Lepidium Sativum* were collected from local area localized in the Northwest of Algeria during May 2012. It was authenticated according to the Flora of Algeria[7].

Plant growth

**Author for Correspondence*

Plants of L. sativum were grown in soil culture with 16/8 h light/dark cycle, 4 weeks old plants were used for the experiment. Our study is performed on the leaves.

Preparation of extracts

Preparation of ethyl acetate and butanolic fractions

The dry seeds and leaves (20 g) powder of *L sativum* was extracted during 48 h by the mixture of methanol and water at 70% (v/v) and filtered throw disks of Watman paper n°1. The process was repeated three times with same quantity of solvent mixture. The different fractions were collected then concentrated in vacuum at 40 °C. The extract was suspended in boiled distilled water (200 mL) and extracted by different solvents (50 mL) in order Hexane, ethyl ether, ethyl acetate and butanol. The different solutions were evaporated and taken up in methanol. Ethyl acetate (rich on O-glycosides) and butanol (rich on C-glycosides) fractions were used for this study. The amount of O-glycoside is expressed as rutine equivalent and C-glycosides content is determined as rhamnetin equivalent.

Preparation of ethyl ether extract

The protocol established by[8] relies on acid hydrolysis of O-glycosides from plant material. The aglycones (flavones and flavonols) were extracted by ethyl ether, dried and then solubilized in methanol. The method used by[9] with slight modifications was followed. The ethyl ether fractions were used to determine Aglycones contents. A differential spectrophotometric assay allowed the flavonoid estimation at 420 nm in the presence of $AlCl_3$. The amount was expressed as quercetin equivalent.

Antitumoral activity

Cell Proliferation Assay

The cells were trypsinised (0.1% of trypsin Gibco, USA) and suspended. After incubation during 48 h, cells were exposed to Aglycones (flavones, flavonols), O-glycosides and C-glycosides during 48 h. The cells were trypsinized and the evaluation of proliferation rate was performed on 100 µL cell suspension by counting on Mallasez cell.

Morphological and morphometic study

The suspended cells have been incubated during 48 h and exposed during 48 h at 57 µg/mL to Aglycones (flavones, flavonols), O-glycosides and C-glycosides. After that, the mediums were eliminated, and the cells were washed with a saline phosphate-buffered (P B S, 1 x) (Gibco), fixed in the aqueous Bouin and colored with May Grumwald–Giemsa (M G G) (V/V, 1/1) and 100 mg/mL orange acridine. The observation was given with an inverted microscope.

Measurement of lipid peroxidation using MDA assay

The MDA level of cells was measured spectrophotometrically. MDA reacts with TBA as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex that has a peak absorbance at 532 nm[10]. The MDA was determinate in the intracellular compartments of control cells during 48 h of incubation and submitted at 57 µg/mL of different extracts. After reaction with thiobarbituric acid TBA (11) cells were homogenized in buffered (Na_2 HPO4 /Na H_2PO4) 0.2 M, pH= 6.5 and centrifuged for 20 mn at 4 °C. The MDA contained supernatant in presence of (10%) TCA reacts with TBA and causes the formation of a complex. The absorbance at 532 nm against a blank (TBA, 1 mL) that contained all reagents except the sample. The amount of MDA equivalents formed was calculated using MDA standard graph prepared under similar reaction conditions.

RESULTS

Evaluation of anticancer activity in vitro

In order to research target molecules, we have evaluated the cytotoxic activity on HEp2 cells. Cancerous cells were exposed to flavones-flavonols, O-glycosides and C-glycosides at 57 µg.mL^{-1} for 24 h and cells treated with DMSO were used as controls (Fig. 1). Microscopic image analysis revealed that all fractions of Lepidium sativum seeds induced the distinct characteristics of apoptosis such as hypertrophic cell, membrane betting, chromatin condensation. The treatment with extract containing O-glycosides was significantly stronger nuclear fragmentation and formation of apoptotic bodies were also appeared. In the case of the C- glycosides treatment, we can observe apoptosis and probably necrosis cells (Fig. 1). The cytotoxic potential of the different fractions of *Lepidium sativum* leaves as also observed. As reported in Fig 1 cells presented very small morphological changes at the same extract concentration. The results showed that aglycones fractions treatment have not provoked the apoptotic effect, the acetate ethyl fraction rich on O-glycosides fraction a little cytotoxicity effect and C-glycosides fraction presented moderated cytotoxicity effect.

Proliferation effect.

As demonstrated in Figure 2, the HEp2 cells proliferation treated with L. sativum seeds extracts were reduced from 87%, 70% and 36% after treatment by ethyl acetate, butanolic and ether ethylique fractions respectively. As shown in Figure 2, the treatment with all extracts obtained from L sativum leaves provoked a decrease on HEp2 cells proliferation. Significant inhibition of cells proliferation more than 78% was observed at 57 µg/ml of extract concentration for ethyl acetate, butanolic and ether ethylique fractions.

Malondialdehyde (MDA) determination

In order to evaluate the ROS accumulation, of treated cells, the malondialdehyde (MDA) were measured as indicator of lipid peroxidation (Fig. 3). The (Fig 3) indicate that only the levels of HEp2 cells MDA treated with glycosides extracts of L. sativum seeds increase with (744%) and (105%) respectively for O-glycosides and C-glycosides comparatively to control. Flavones/ flavonols do not affect MDA levels (Fig. 3). After treatment with ethyl acetate we have observed apoptotic cells, and MDA HEp2 cells measurements is highly increased .it indicate that there is an accumulation of free radicals. which take part in the signaling mechanism of appoptosis. This results clearly suggest that the effect of ethyl acetate L. sativum seeds fraction is due to the induction of apoptosis. Significant inhibition of proliferation cells of more than 78% was observed after treatment with all L;sativum leaves extracts. Whereas a negligible effect was shown on HEp2 cells MDA content. the decrease of proliferation cells was probably not due to the apoptosis phenomena.

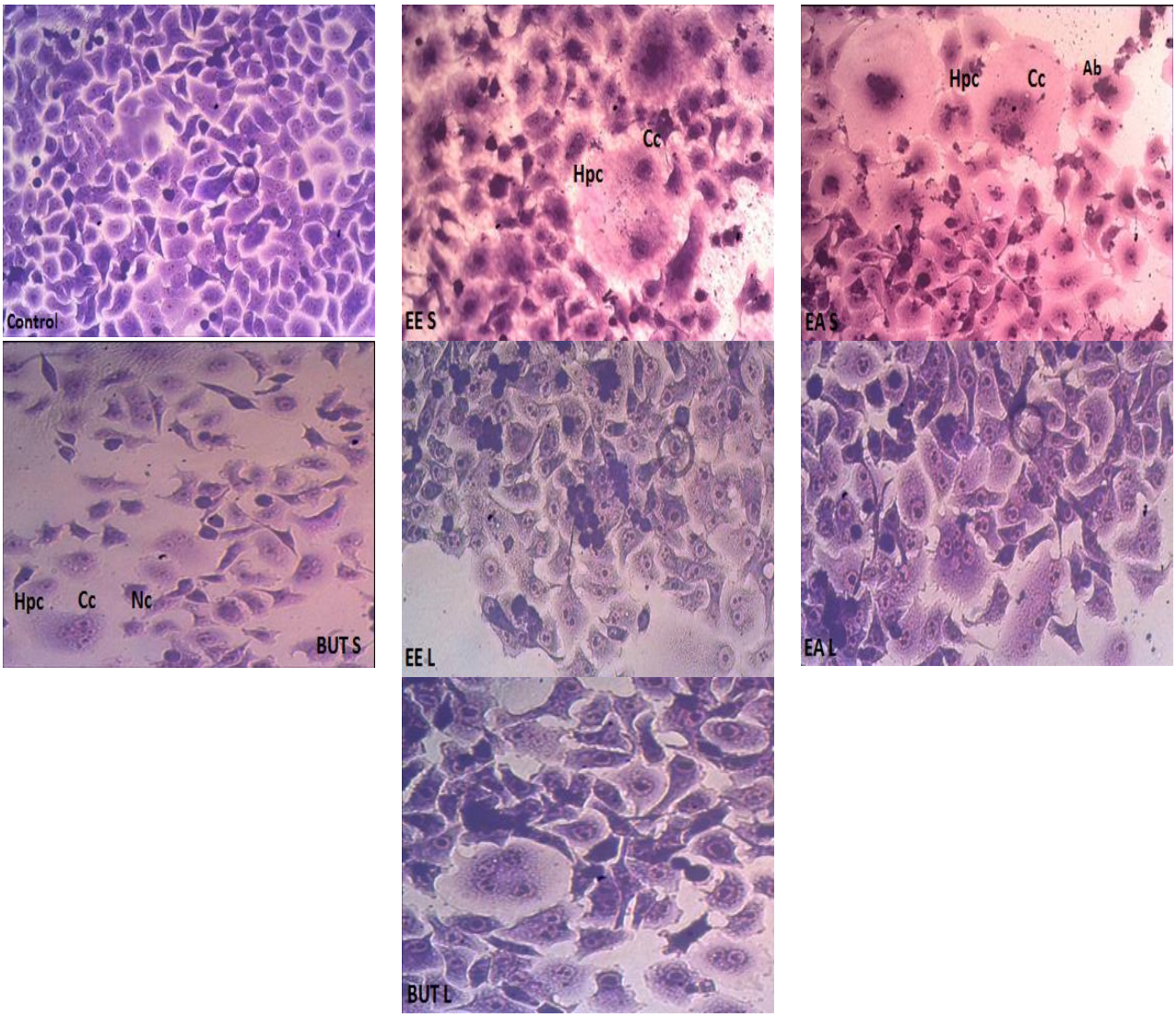

Figure 1. Effects of ethyl ether (Aglycones,) ethyl-acetate (O-glycosides) and butanolic (C-glycosides) fractions from L. sativum seeds and leaves at 57 µg/ mL on the morphology of Human Laryngeal Carcinoma cells **(**HEp 2) after 48 h of treatment. The cells were colored with May Grumwald–Giemsa (M G G) (G X 86,95 µm) **EE S:** ethyl ether seeds fraction **(**Aglycones), **AE S:** ethyl-acetate seeds fraction (O-glycosides), **BUT S:** butanol seeds fraction (C-glycosides), **EE L**: ethyl ether leaves fraction **(**Aglycones), **AE L:** ethyl-acetate leaves fraction (O-glycosides), **BUT L:** butanol leaves fraction (C-glycosides), **Hpc:** Hypertrophic cell, **Ab:** Apoptotic bodies, **Cc:** Chromatin condensation, **Nc:** Necrosis cell.

DISCUSSION

Proliferation inhibition and apoptotic induction of tumor cells are phenomena to prevent tumor growth and to eliminate cancer all extracts tested in the present study possessed antitumor activities towards Hep2. These results have demonstrated clearly that the extract from L. sativum seed containing O-glycosides (ethyl acetate fraction) induced an important inhibition of tumor cells than C-glycosides and aglycones. It seems that these compounds act differently on HEp2 cells. The O-glycosides provoked increase in MDA content which is an indicator of lipid peroxidation. Accumulation of Reactive Oxygen Species (ROS) in cell caused the peroxidation lipid. The ROS are important chemical messengers to promote apoptosis phenomena[12]. In the case of aglycones, we have observed the apoptosis effect but not accumulation of the ROS.

It could be explained by a different signaling cascade probably by cell cycle arrest. Oxidative stress and cell-cycle regulation are two essential elements in the apoptosis process. Also, another form of cellular death was observed after C-glycosides treatment[13]. have demonstrated that O-glycosides obtained from *Gleditsia triacanthos* were inactive at different concentrations (12.5 - 100 µg/mL) towards Hep G2.Whereas, the vitexin (C-glycosides) at 100 µg/mL induce the lowest effect. Compared to the previous report, we have found that *Lepidium sativum* extracts exhibit a significant cytotoxic activity. In contrast the different extracts from L. sativum leaves causes an important decrease of proliferative toward HEp2 cells (more than 78%), this effect is not associated with an induction of apoptosis. This results is probably due to the composition of *L sativum* leaves flavonoids, Flavonoids of *Brassicaceae* (=*Cruciferae*) plants are present in high

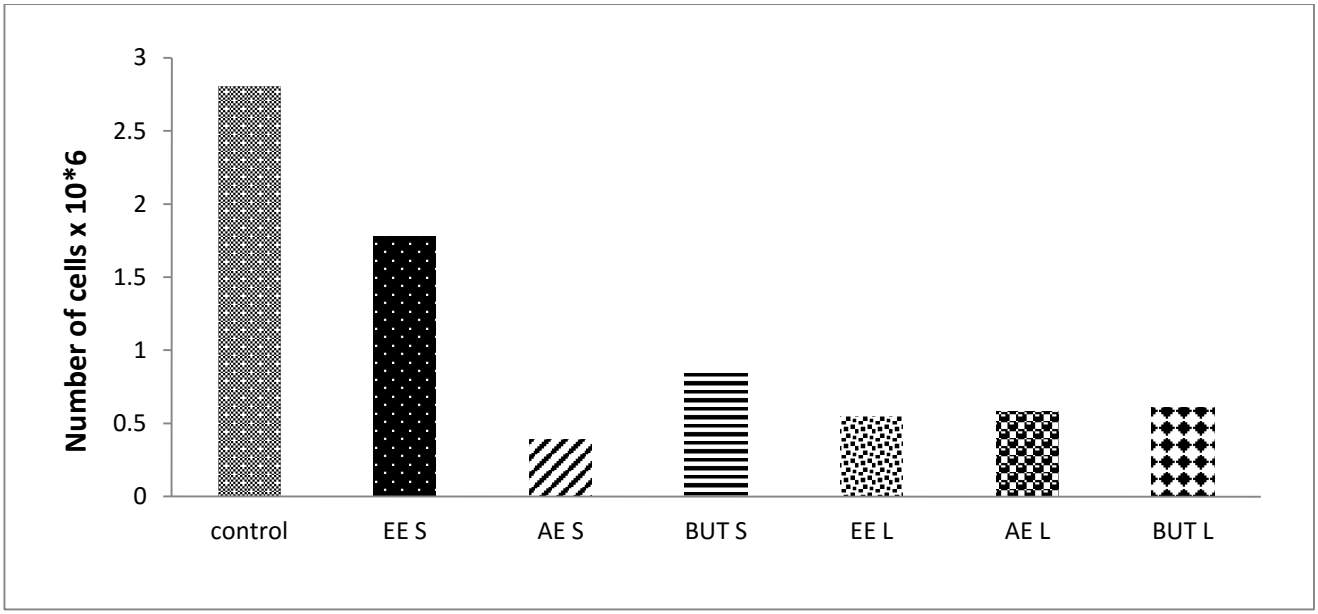

Figure 2. Proliferation effect on HEp 2 cells of different flavonoid extracts.
EE S: ethyl ether seeds fraction **(**Aglycones), **AE S:** ethyl-acetate seeds fraction (O-glycosides), **BUT S:** butanol seeds fraction (C-glycosides), **EE L**: ethyl ether leaves fraction **(**Aglycones), **AE L:** ethyl-acetate leaves fraction (O-glycosides), **BUT L:** butanol leaves fraction (C-glycosides)

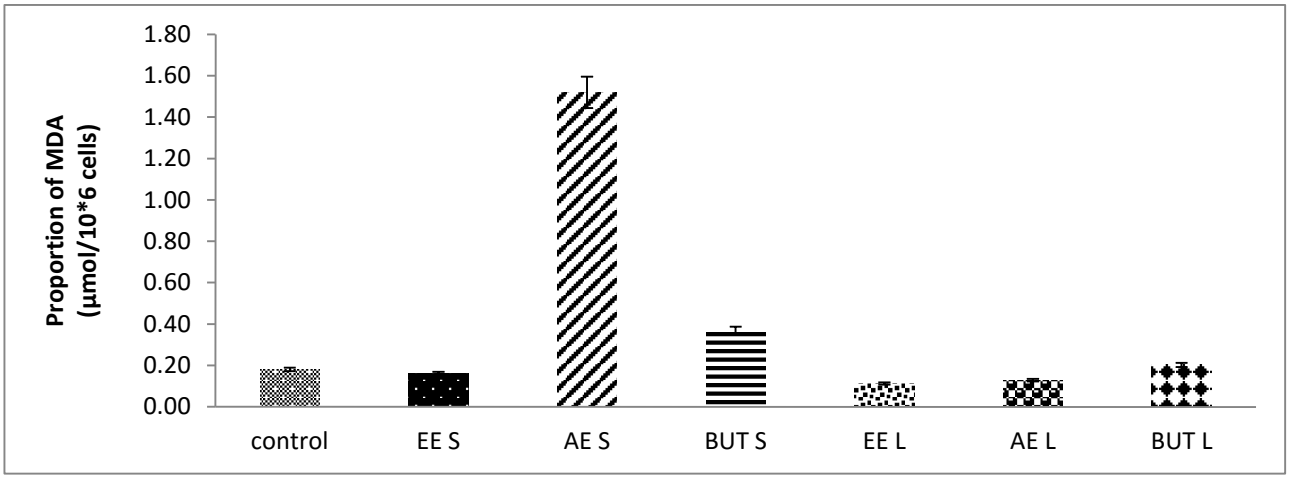

Figure 3 The MDA proportion of various antioxidant compounds (Aglycones, O-glycosides and C glycosides).
EE S: ethyl ether seeds fraction **(**Aglycones), **AE S:** ethyl-acetate seeds fraction (O-glycosides), **BUT S:** butanol seeds fraction (C-glycosides), **EE L**: ethyl ether leaves fraction **(**Aglycones), **AE L:** ethyl-acetate leaves fraction (O-glycosides), **BUT L:** butanol leaves fraction (C-glycosides)

concentrations in the epidermis of leaves and fruits. Flavonols are the most widespread of the flavonoids. quercetin, kaempferol and isorhamnetin, are the main flavonols in *Brassicaceae* species, and they are most commonly found as *O*-glycosides[5]. L. sativum possessed also quercetin, kaempferol[14] A variety of indirect anticancer effects of flavonoids have been reported, including inhibition of cancer cell proliferation and induction of apoptosis[15]. Quercetin reported suppress the proliferation of cancer cells but did not affect normal cells[16,17]. kaempferol (flavonol) inhibited proliferation of malignant human cancer cell lines[18].

CONCLUSION

Flavonoids from L. sativum seeds and leaves were investigated for their cytotoxic activities towards HEp2 cells.

The results obtained in this study showed that ethyl acetate fraction (O-glycosides) of *L. sativum* seeds had the best cytotoxic effect towards HEp2 cells followed by butanol seed fraction . We report the first study on flavonoids of L. sativum leaves, we can conclude that all extracts possess a good cytotoxic activity. Additional prospective studies are required to confirm these findings, in first time to isolate and identificate the responsible compound of this cytotoxicity. And in another hand, these extracts could be evaluated toward different types of cancer.

ACKNOWLEDGEMENT

The authors are grateful to Dr Haj-Arab. H. for the identification of the plant and thank also Asli-Amalou for her help.

REFERENCES

1. Wadhwal S, Panwar MS, Agrawal A, Saini N, Patidar LN. A review on pharmacognostical study of *Lepidium sativum.* Advance Reseaarch in Pharmaceuticals and Biologicals 2012; 2: 316-323.
2. Manohar D, Viswanatha GL, Nagesh S, Jain V, Shivaprasad HN. Ethnopharmacology of *Lepidium Sativum* Linn (Brassicaceae): A review. Int. J. Phyt. Res 2012; 2: 1–7.
3. Mahassni SH, Al-Reemi, RM. Apoptosis and necrosis of human breast cancer cells by an aqueous extract of garden cress (*Lepidium sativum*) seeds. SAUDI Journal of Biological Sciences 2013; 20: 131-139
4. Fan QL, Zhu YD., Huang WH, Qi Y, Guo BL. Two new acylated flavonol glycosides from seeds of *Lepidium sativum*. Molecules 2014; 19: 11341-11349.
5. Cartea ME, Fransisco M, Soengas P, Velasco P. Phenolic compounds in *Brassica* vegetables. Molecules 2011; 16: 251-280.
6. Xiao J, Muzashviti TS, Georgiev MI. Advances in the biotechnological glycosylation of valuable flavonoids. Biotechnology Advances 2014; 32: 1145-1156.
7. Quezel P, Santa S. Nouvelle flore de l'Algérie et des régions désertiques méridionales, vol. 1, Éditions du Centre national de la Recherche scientifique, Paris, France, 1962, 406.
8. Lebreton P, Jay M, Voirin B. Sur l'analyse qualitative et quantitative des 14 flavonoïdes. Chimie Analytique Paris 1967; 49: 375-383.
9. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoids contents in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10: 178-182.
10. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 1968; 125: 189-198.
11. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biology and Medicine 1990; 9: 515-540.
12. Hwang YJ, Lee EJ, Kim HR, Hwang KA. Molecular mechanisms of luteolin-7-O-glucoside-induced growth inhibition on human liver cancer cells: G2/M cell cycle arrest and caspase-independent apoptotic signaling pathways. BMB Reports Online 2013; 46: 611-616.
13. Mohammed RS, Zeid AH, El-hawary SS, Saleem AA, Ashour WE. Flavonoid constituents, cytotoxic and antioxidant activities of *Gleditsia triacanthos* L. leaves. Saudi Journal of Biological Sciences 2014; 21: 547-533.
14. Jyoti A, Verma DL. Antioxidative Activity and Flavonoid Composition from *Lepidium sativum*. Nature and Science 2011; 9(7):21-25.
15. Fantini M, Benvenuto M, Masuelli L, Frajese GV, Tresoldi I, Modesti A, Bei R. Review In Vitro and in Vivo Antitumoral Effects of Combinations of Polyphenols, or Polyphenols and Anticancer Drugs: Perspectives on Cancer Treatment. International Journal of Molecular Science 2015; 16: 9236-9282.
16. Soleas GJ, Grass L, Josephy PD, Goldberg DM, and Diamandis EP. A comparison of the anticarcinogenic properties of four red wine polyphenols. Clin Biochem; 2002 35(2): 119–124.
17. Granado-Serrano AB, Martín MA, Bravo L, Goya L, Ramos S. Quercetin modulates NF-kappa B and AP-1/JNK pathways to induce cell death in human hepatoma cells. Nutr. Cancer 2010; 62(3): 390–401.
18. Cho YY, Yao K, Pugliese A, Malakhova ML, Bode AM, and Dong Z. A regulatory mechanism for RSK2 NH(2)-terminal kinase activity. Cancer Res 2009; 69: 4398–4406.