American Journal of Biochemistry and Biotechnology, 9 (2): 102-117, 2013
ISSN: 1553-3468

doi:10.3844/ajbbsp.2013.102.117 Published Online 9 (2) 2013 (http://www.thescipub.com/ajbb.toc)

Antioxidant and Antimutagenic Activities of Taif Grape (*Vitis vinifera*) Cultivars

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Received 2013-01-31, Revised 2013-04-15; Accepted 2013-04-30

ABSTRACT

Extract of grape *Vitis vinifera* has been reported to exhibit antioxidant and antimutagenic activities and the phenolic compounds play a vital role in determining these activities. Therefore; the objective of the present study was to evaluate the antioxidant and anti-mutagenic activities as well as the phenolic composition of different grape cultivar extracts collected from Taif region. The grape cultivars namely; Italian, American, Lebanese, $Taify_b$ and $Taify_e$ were collected at maturity stage to represent Taif region cultivars. The total concentrations of phenoles were determined for the five cultivar extracts and results indicated that the concentrations ranged from 115-960 mg L^{-1} Gallic Acid Equivalent (GAE). Also, HPLC analysis included was carried out of nine important phenolic compounds namely; Cyanidine chloride, Myricetin, Chrysin, Quercetin, Delphinidine chloride, Malvidine chloride, Naringenin, Galangin and Caffeic acid. Significant differences among cultivars were obtained for each compound. However, the highest cultivar for each compound differed from compound to another. At the same time, DPPH was used to estimate antioxidant activity and the data showed that different grape cultivar extracts were able to quench 47-60% of DPPH radical solution and to exhibited potent radical scavenging activity. Also, antimutagenic activity was measured as a decrease of chromosomal aberrations in bone marrow cells of mice treated with the mutagen Endoxan. Results showed that treatment of mice with grape cultivar extracts resulted in a significant decrease in all types of chromosomal aberrations induced by Endoxan. Also, the anticlastogenic effect was measured using micronulei test and results indicated that all grape cultivar extracts reduced significantly the effect of Endoxan on micronulei test. Finally, treatment of mice with grape cultivar extracts enhanced mitotic index of mice bone marrow cells reduced by Endoxan treatment. The relationship between phenolic compound concentrations and antioxidant capacity was discussed.

Keywords: Micronuclei Test, Total Phenols, *Vitis vinifera*, DPPH, Chromosomal Aberrations

1. INTRODUCTION

Free radicals are normally generated in substantial amounts as a by-product of various internal metabolic processes and they can also be generated in the human body during microbial infection and lipid peroxidation (Valko *et al*., 2007). There is now overwhelming evidence to indicate that free radicals causing oxidative damage to lipid, protein and nucleic acid. However, humans have evolved highly complex antioxidant systems (enzymic and nonenzymic), which work synergistically and in combination with each other to

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protect the cells and organ systems (Sadaf *et al*., 2012) Therefore, it is important to obtain antioxidant exogenously as a part of a diet or as dietary supplements (Sadaf *et al.*, 2012). An ideal antioxidant should be readily absorb and quench free radicals and chelate redox metals at physiologically relevant levels. Endogenous antioxidants play a crucial role in maintaining optimal cellular functions and thus systemic health and well-being. However, under conditions, which promote oxidative stress, endogenous antioxidants may not be sufficient and dietary antioxidants may be required to maintain optimal cellular functions.

Grapes *Vitis vinifera* and grape products have an economic value where they are mostly consumed as table grapes, grape juice or raisins. Extract of grape has been reported to exhibit antioxidant activity, scavenging both free radicals and reactive oxygen species both in vivo and in vitro. Also, it is well established that phenolic compounds play a vital role in determining the grapes antioxidant activity. The amount of total phenolics content, which expressed as mg GAE/g was 150.69 mg GAE/g in red grapes from Saudi Arabia local market (Qusti *et al*., 2010). The phenolic substances are primarily located in the seeds and skins of the berry, (Ali *et al*., 2010). Grapes contain a large number of different phenolic compounds in skins, pulp and seeds especially anthocyanins, catechins and oligomeric proanthocyanidins which partially extracted during juice-making (Revilla and Ryan, 2000). At present, several hundreds of phenolic compounds from grapes have been identified. These phenolic compounds were found to exhibit antioxidant activity in vivo and in vitro (Sakkiadi *et al*., 2001). The highest values of antioxidant activity, inhibition of low-density lipoproteins and total polyphenols were determined in pomace, grapes and must (Yildirim *et al*., 2005). One of these compounds is caffeic acid which is one of the most common hydroxycinnamate acids in grape juice. Caftaric acid, the most abundant hydroxycinnamate found in grapes, consists of caffeic acid bound to tartaric acid. Caftaric acid is hydrolysed naturally in juice (Waterhouse, 2002) liberating caffeic acid. The content of caffeic acid in grapes can be as high as 70 mg L^{-1} (Makhotkina and Kilmartin, 2010). Antioxidant activity of caffeic acid was established (Kurin *et al*., 2012).

Flavonoids constitute the majority of phenolic compounds (65-76%) in grapes and anthcyanins are the major group of the favonoids (Hogan *et al*., 2009). However, the major dietary flavonoids are often classified under six groups. Anthocyanidins (e.g., delphinidin, cyanidin, petunidin, peonidin and malvidin). Flavonols (e.g., quercetin, kaempferol and quercetagetin). Flavanols (also called proanthocyanidins, flavan-3-ols or catechins, e.g., catechin, epicatechin, epicatechin gallate and epigallocatechin-3-gallate). Isoflavonoids (isoflavones, e.g., genistein, diadzein, formononetin and biochanin A and coumestans, e.g., coumestrol). Flavones (e.g., rutin, apigenin, luteolein and chrysin). Flavanones (e.g., myricetin, hesperidin, naringin and naringenin) (Peterson and Dwyer, 1998). Flavonoids have been reported to have *in vivo* and *in vitro* antioxidant activities through their ability to scavenge the radicals of hydroxyl, peroxyl, superoxide, nitric2 oxide and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Awah and Verla, 2010).

Besides berries, a relevant part of intake of polyphenolic including flavonoids is supplied by fruit juices. Juices are suitable food products in terms of ingestion of health protective phytochemicals. Bioactive components may even be better absorbed from juices than from plant tissues, as it was demonstrated for ascorbic acid (Netzel *et al*., 2005; 2007). Epidemiological studies and associated meta-analyses strongly suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Pandey and Rizvi, 2009). There is still not enough knowledge about health effects of fruits, vegetables juices or antioxidant concentrates. Many reports have been written about the antioxidant activity and flavonoids profiles of various fruits or fruit extracts (Zhou and Raffoul, 2012), but few relatively have been based on fruit juices. A need for such data still exists because of increasing popularity of fruit juice consumption during the last time and because of increasing consumer awareness concerning the nutritional value of all foods including these juices.

Research on antioxidant activity and phenolic content of Taif region grape cultivars is rare. Only one report had been made by Qusti *et al*. (2010). They investigated antioxidant capacity of a number of fruits, vegetables and grains including grapes based on their ability to scavenge (DPPH) stable free radical. Also, they determined the phenolic content of Taif white grape extracts. Their results showed a good correlation between antioxidant activity and phenolic content. Therefore; the objective of the present study is to evaluate the antioxidant and anti-mutagenic activities as well as the phenolic composition of different grape cultivar extracts collected from Taif region.

2. MATERIALS AND METHODS

2.1. Plant Material

Italian, American, Lebanese and two clones of Taify table grape cultivar were selected from different locations in Taif governorate (**Table 1**). Three replicates for each cultivar were taken. Three clusters from each cultivar were harvested, immediately transported to the laboratory and split from bunches and frozen at -25°C until analyzing. About fifty of frozen grapes berries per each cultivar were thawed overnight (12 h) at 4°C and crushed using a blender and allowed to settle to obtain a clear juice. Juice was filtered on a double layer cheese cloth to remove skin and pulp from the juice. Crushing of grapes, juice extraction and filtration were performed in a cold room maintained at 3±1°C. The juice obtained was immediately frozen at -25°C. Frozen juice samples were analyzed within one month of juice preparation.

2.2. Chemicals

Endoxan (cyclophosphamide) was obtained from Asta Medica AG, Frankfurt, Germany. Vitamin C, 1,1-diphenlyl-2-picrylhidrazyl (DPPH) and nine chemical standards (Cyanidine chloride, Myricetin, Chrysin, Quercetin, Delphinidine chloride, Malvidine chloride, Naringenin, Galangin and Caffeic acid) were purchased from Sigma-Aldrich.

2.3. Animals

Seven-weeks-old male Swiss albino mice (*Mus muscullus*, 2n = 40) weighing about 25 g were used. The animals were reared in poly-propylene cages and were maintained at 28°C. Mice were fed with standard mouse pellets composed of 33% berseem hay (*Trifolium alexandrinum*), 17% soybean meal, 16.5% ground corn, 16% barley, 12% wheat bran, 3.8% molasses, 1% salt, 0.4% dicalcium phosphate and 0.3% vitamins. All animals were allowed for a suitable period to adjust to the new environment before the onset of the experiment (Salama *et al*., 1995)

2.4. Total Phenols

Total phenols was measured using the method mentioned in Fahmi *et al*. (2011). 20 uL from each sample or blank was pipeted into cavette and to each 1.58 ml ddH2O, 100 uL of Folin-Ciocalteu reagent were added. After 8 min, 300 uL of Na2CO3 (20%) was added. Solution was left for 2hr and absorbance was measured at 765 nm. Calibrated curve with standard solution of gallic acid concentrations 0, 50, 100, 150, 250, 500 and 1000 mg L^{-1} gallic acid was prepared. Finally, the concentrations of phenols in samples were determined and results were reported at gallic acid equivalent.

2.5. HPLC Analysis

HPLC analysis was performed on an Agilent HP 1100 system (Agilent Technology, Palo Alto, CA). HPLC analysis was conducted according to method described by Pietta *et al*. (2002). The grape juices were analyzed for the levels of individual polyphenols using an HPLC Aglient 1100 (Fahmi *et al*., 2012). HPLC standards (Cyanidine chloride, Myricetin, Chrysin, Quercetin, Delphinidine chloride, Malvidine chloride, Naringenin, Galangin and Caffeic acid) were dissolved in ethanol at a final concentration of 1 mg mL^{-1}. Hewlett-Packard Phenomenex Luna C18 (4.6×250 mm, 10 µm particle size) column was used. Eluents used were ammonium acetate (100 mM, pH 5.5) as eluent A and methanol absolute HPLC grade as eluent B. The elution program was started at 100% of eluent A and end with 100% of eluent B in 13 min duration as follows: Starting condition with 100% eluent A, 0-3 min reaching 70% eluent A and 30% eluent B, 3-8 min reaching 50% eluent A and 50% eluent B and 8-13 min reaching 0% eluent A and 100% eluent B. Flow rate used was 1.5 mL min^{-1} with oven temperature of 30°C. The injected volume of sample and standards was 20 µL. The column eluate was monitored at 260 nm. The individual polyphenols were quantitated by comparing HPLC retention times with known amounts of standards. All concentrations were measured in µg/mL.

2.6. DPPH Scavenging Activity

Scavenging effect of grape samples corresponding of the quenching intensity of 1,1-diphenlyl-2-picrylhidrazyl was carried out mentioned in (Fahmi *et al*., 2011). Sample solution of each tested material (500 uL) was mixed with the same volume of 60 uM of DPPH solution and was allowed at dark for 30 min at room temperature. The absorbance was measured at 517 nm. The percentage of scavenging effect was determined by comparing the absorbance solution containing the test sample to that of blank sample as follows:

$$\% \text{ DPPH Scavenging activity} = \left(A_0 - A_1\right) / A_0 \times 100\%$$

Table 1. Grape (*Vitis vinifera* L.) cultivars understudy and their locations in Taif's governorate, Saudi Arabia

Cultivar	Location
Italian	Prince Bandar Farm, Al roddaf region, Taif
American	Prince Bandar Farm, Al roddaf region, Taif
Lebanese	Prince Bandar Farm, Al roddaf region, Taif
$Taify_b$ ($clone_b$) (Al-Bayadi)	Mastour Farm, Al Raha village, Al wadi region
$Taify_e$ ($clone_e$) (Al-Bayadi)	Al Raha village, Al Khowkaa region

Where:
A_0 = Measurement of the blank
A_1 = Measurement of the sample

2.7. Antimutagenic Effect

Grapes samples and Vitamin C (VC) were used as antimutagenic agents. Eighty male mice were randomly distributed into eight groups of 10 animals each and were assigned at random to one of the following treatments. Positive Control chemical and antioxidant agents (PC, VC) were pre administered orally using micropipette before meals. The first group served as Negative Control (NC) and was treated with water during the period of the experiment (6 days). Second group served as Positive Control (PC) and were given Endoxan (positive control mutagene) at concentration of 25 mg kg^{-1} of mice for 6 days (Fahmi *et al*., 2011).

Third group (VC) was given 20 mg kg^{-1} of Vitamin C for six days and Endoxan for the last 3 days of the experiment. The rest of the groups were given the grapes extract for six days and were treated with Endoxan during the last three days of experiment at 25 mg kg^{-1}.

Animals were sacrificed by decapitation 24 h after the last dose of all treatments. Three hours prior to killing, animals were injected with 0.6 mg kg^{-1} of colcimed after killing, the adhering soft tissue and epiphyses of both tibiae were removed. The marrow was aspirated from the bone, transferred to phosphate buffered saline and centrifuged at 1000 rpm for 5 min. Pellets were resuspended in 0.075 M KCl. Centrifugation was repeated and the pellet was resuspedned in fixative (methanol: acetic acid, 3:1). The fixative was changed after 2 h and the cell suspension was left overnight at 4°C.

Bone-marrow smears were made according to Fahmi *et al*. (2011). Cells in fixative were dropped on very clean glass slides and air-dried. The slides were fixed in absolute methanol for 5 min, rinsed twice in deionized distilled water, stained for 5-10 min in 10% Giemsa at pH 6.8 for 5 min. Finally, slides were coded and screened for Chromosomal Aberrations (CAs); stickiness, chromatid gap, Robertsonian Centric Fusion (RCF) and deletion (1500 divided cells were investigated per treatment).

Also, micronucleus test was conducted as mentioned in (Fahmi *et al*., 2011). Peripheral blood smears were prepared and slides were air-dried for 24 h, fixed in methanol for 1 min, followed by 10% Giemsa (v/v) staining. Four hundred erythrocytes were examined for each animal (4000 per treatment). Micronuclei were identified as dark-blue staining bodies in the cytoplasm of polychromatic erythrocytes. The frequency of micronucleated cells was expressed as a percent of total erythrocytes investigated. Finally, anticlastogenic index was calculated as follows:

$$ACI = 100 - \left\{ \begin{array}{l} (\%\ \text{MN of tested compound}) \\ -(\%\text{MN of NC}) / \\ (\%\text{MN of PC clastogen}) - (\%\text{MN of NC}) \end{array} \right\} \times 100$$

Where:
MN = Micronuclei
NC = Negative control
PC = Positive control

At the same time, a group of five animals were used for mitotic index assay therefore; they were not injected with colcemid. A mitotic index based on at least 4000 counted cells was recorded. The mitotic activity was estimated as the percentage of dividing cells to the total number of the examined cells (Fahmi *et al*., 2011).

2.8. Statistical Analysis

One-way ANOVA followed by Duncan's multiple range test DMRT or Least significant difference test LSD was used to assess the statistical significance of changes in all indices with the level of significant difference set at $p<0.05$. Statistical analysis software (SPSS 16.0.0 release; SPSS Inc., Chicago, IL) was used for all analyses.

3. RESULTS

This study was obtained to address a principal question concerning the health benefits of the red grapes as antioxidant and as an antimutagenic agent. Five grape cultivars used for this study were grown at the vineyards of different locations in Taif region (**Table 1**). Grapes cultivars were collected at maturity stage and samples were chosen to represent Taif region cultivars. The sampling was randomly made by picking berries from the top, central and bottom parts of the clusters. The juices were obtained from the berries of the cultivars.

3.1. Total Phenols

In this study, a procedure based on the reported method (Fahmi *et al*., 2011) was used with some modifications based on testing the effects of temperature and time of the reaction between Folin-Ciocalteu reagent and standard solutions of gallic acid. Therefore, total phenols were measured in five cultivars as Gallic Acid Equivalent (GAE). The results obtained from the test, for all cutlivars understudy are presented in **Table 2**. As can be seen from **Table 1**, The Italian cultivar had the highest value, Labanese was the second and $Taify_b$ showed the lowest value. Significant differences among cultivars were noticed. The phenols concentrations ranged from 115 - 960 mg L^{-1} (GAE).

3.2. HPLC Estimation of Phenolic Compounds

The HPLC chromatograms of fruit juices recorded at 260 nm are presented in **Fig. 1 and 2** and **Table 3**. Results showed that the american and $Tafy_b$ contained the nine compounds understudy. Italian cultivar does not contain delphinidine, Lebanese does not contain the nonflavonoid caffeic acid and $Tafy_c$ does not contain cyanidine. Also, results showed that the Labanese cultivar contained the highest value of total of these phenolic compounds (12.36 ug mL^{-1}), the second was the Italian cultivar and the lowest was $Taify_c$ (**Table 3**, **Fig. 1 and 2**). The differences among these cultivars for the total of these nine phenolic compounds were significant. At the same time, significant differences among cultivars were obtained for each compound. However, the highest cultivar for each compound differed from compound to another. For cyanidine the Labanese was the first, for myricetin was $Taify_b$, for quercetin was Italian, for chrysin was Italian, for caffeic acid was Italian, for delphinidine was $Taify_e$, for malvidine was Labanese, for naringenin was Italian and for galangenin was Italian. Also, the main compound in each cultivar was different for example in Italian cultivar was caffeic acid while in Labanese cultivar was malvidin chloride.

3.3. Antioxidant Activity

In this assay, results are expressed as the ratio percentage of the absorbance decrease of DPPH radical solution in the presence of grapes at 517 nm to the absorbance of DPPH radical solution at the same wavelength (**Table 4**). The data showed that different grape cultivars were able to quench 47-60% of DPPH radical solution and to exhibited potent radical scavenging activity. The strongest radical scavenging activity showed by Lebanese cultivar while, the American showed the lowest value for inhibition of DPPH. Significant differences were noticed among all cultivars for antioxidant activity.

3.4. Antimutagenic Activity

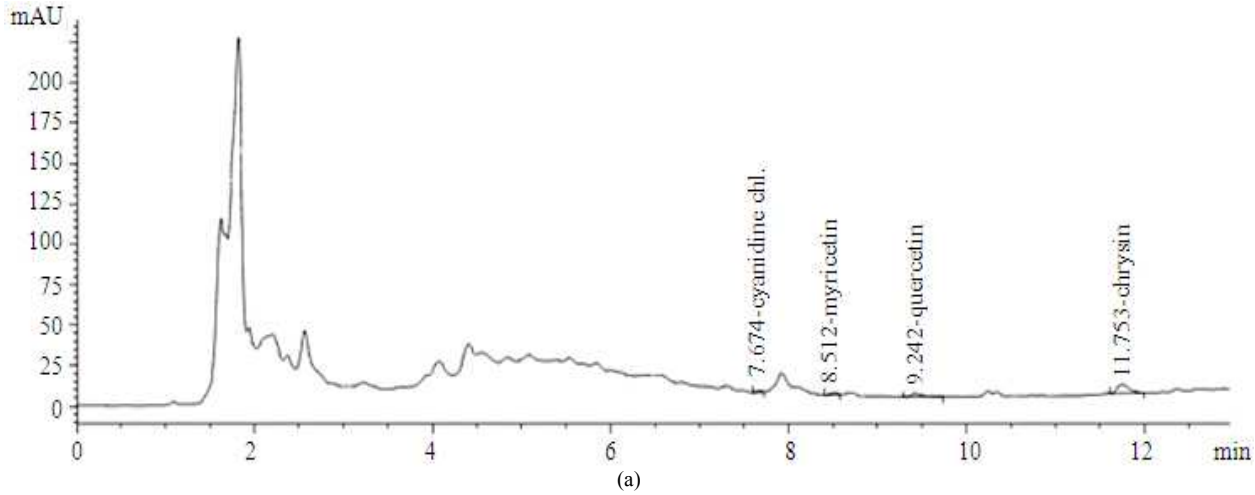
Treatment of mice with endoxan alone showed a high effect on all types of aberrations (**Table 5 and 6, Fig. 3 and 4**), while treatment with Endoxan and vitamin C the positive antimutagenic decreased the aberrations. Also, treatment with different cultivars of grapes decreases the effect of Endoxan. The effect of Vitamin C was higher than any other grape cultivar extracts. The Italian cultivar showed the highest effect among cultivars while Taifye showed the lowest effect.

Treatment of mice with grape cultivar extracts resulted in a significant decrease in all types of Chromosomal Aberrations (CAs). Treatment with Vitamin C along with Endoxan as potent antioxidant significantly reduced all CAs. Treatment with grape cultivar extracts has similar effects to that of treatment with VC. Treatment with grape cultivar extracts after the treatment with Endoxan (T1) did not eliminate the mutagenic effect of Endoxan completely as in the negative control group, but it set it back to insignificant levels of VC effect.

Table 2. Total phenols content of grape (*Vitis vinifera* L.) cultivars understudy expressed as Gallic Acid Equivalent (GAE)

Cultivar	Total phenols mg/L (GAE)
Italian	960[a]
American	365[b]
Lebanese	920[c]
$Taify_b$	115[d]
$Taify_e$	385[e]

*; Values within a column followed by the same letter (s) are not significantly different at the p = 0.05 level according to the DMRT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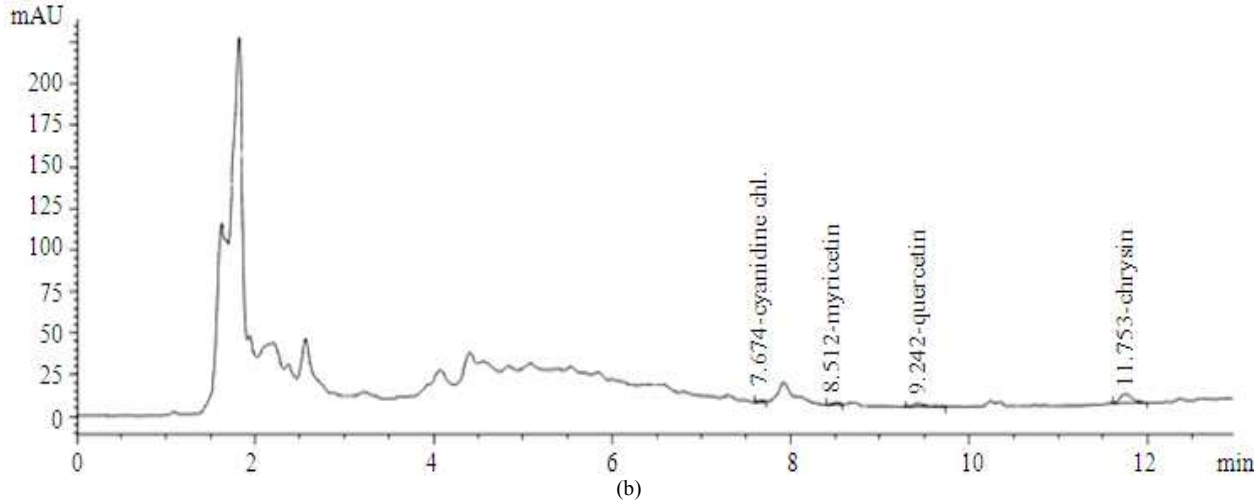
12
min

(a)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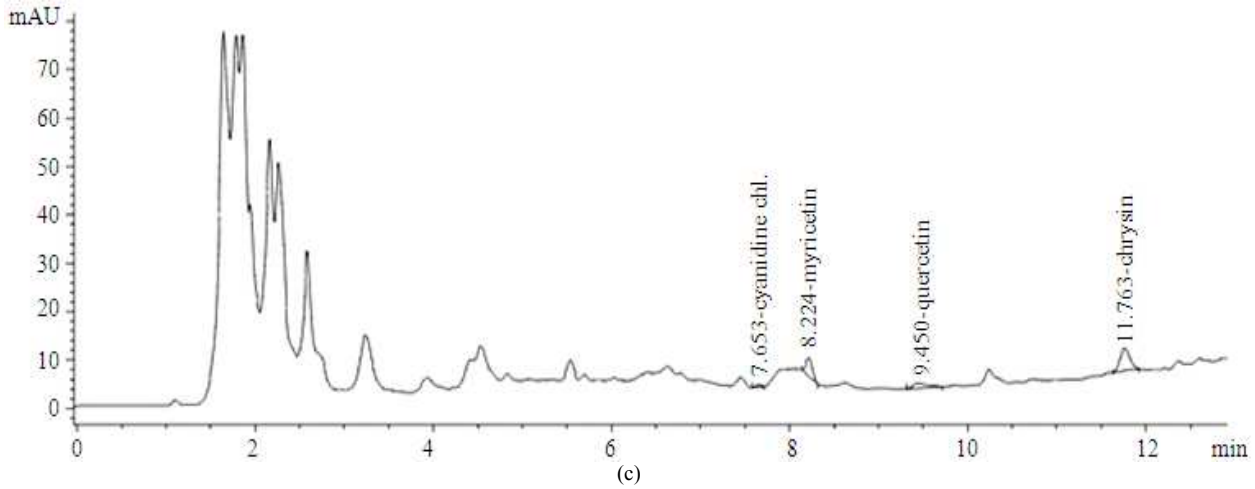
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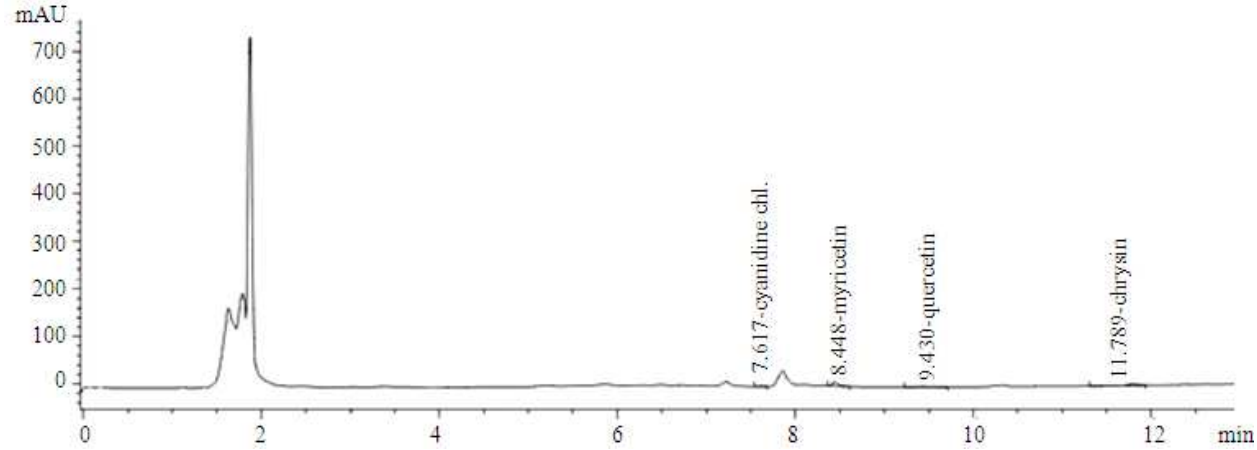

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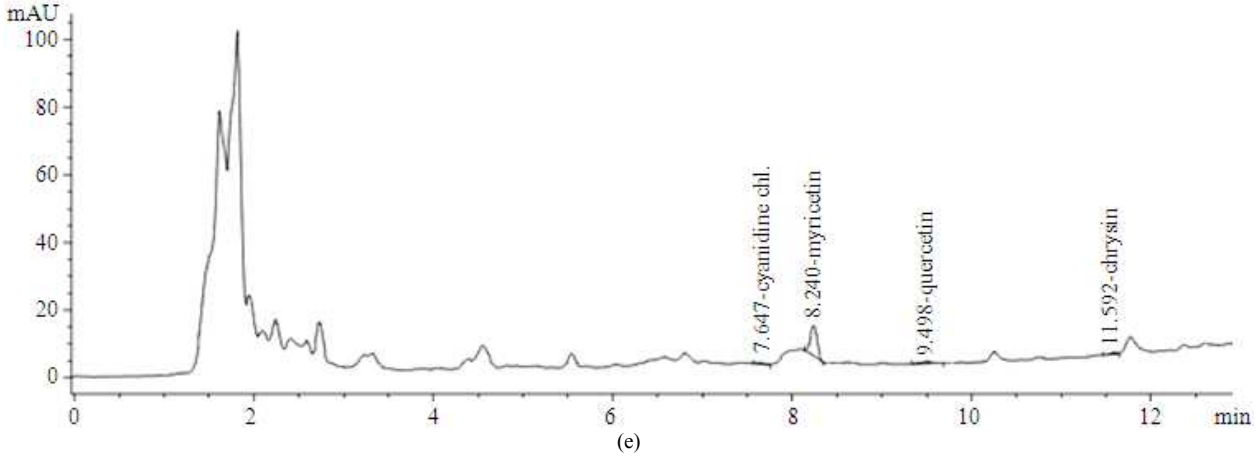

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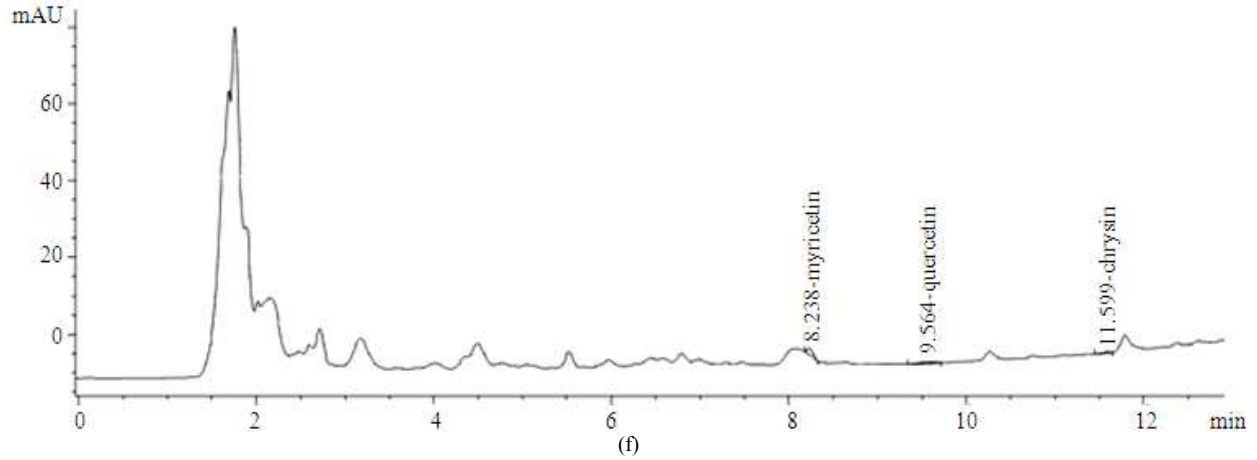
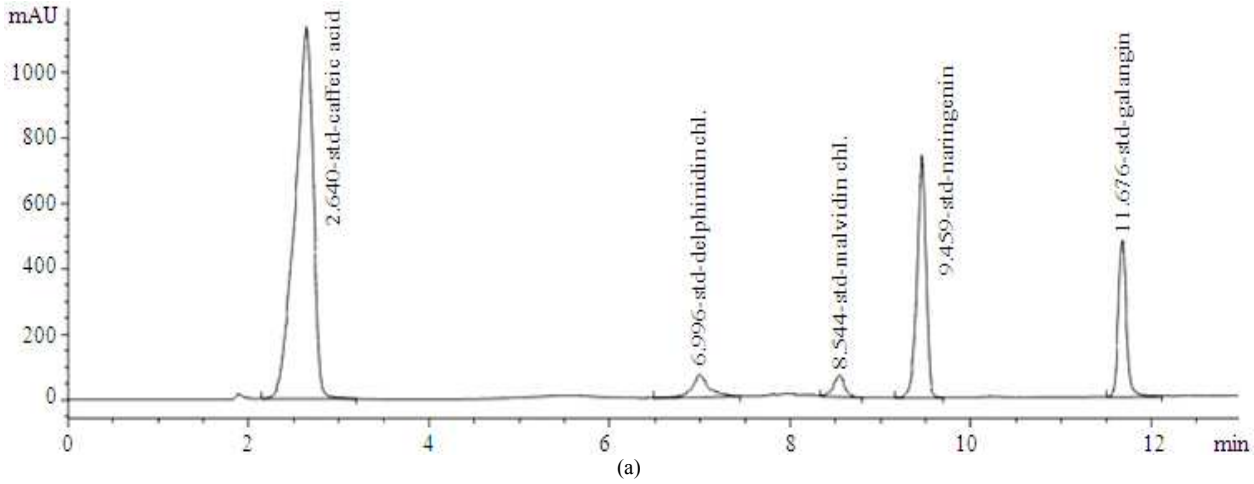


(f)

Fig. 1. HPLC analysis of (a) Cyanidine chloride, Myricetin, Quercetin and Chrysin standards mix, (b) Italian cultivar, (c) American cultivar, (d) Lebanese cultivar, (e) $Taify_b$ and (f) $Taify_e$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8.544-std-malvidin chl.
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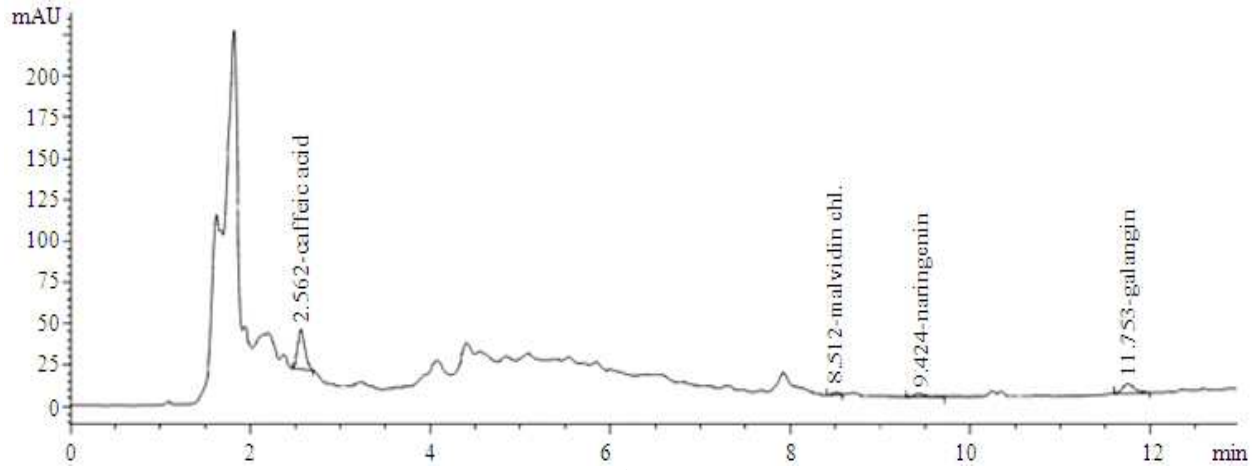
(a)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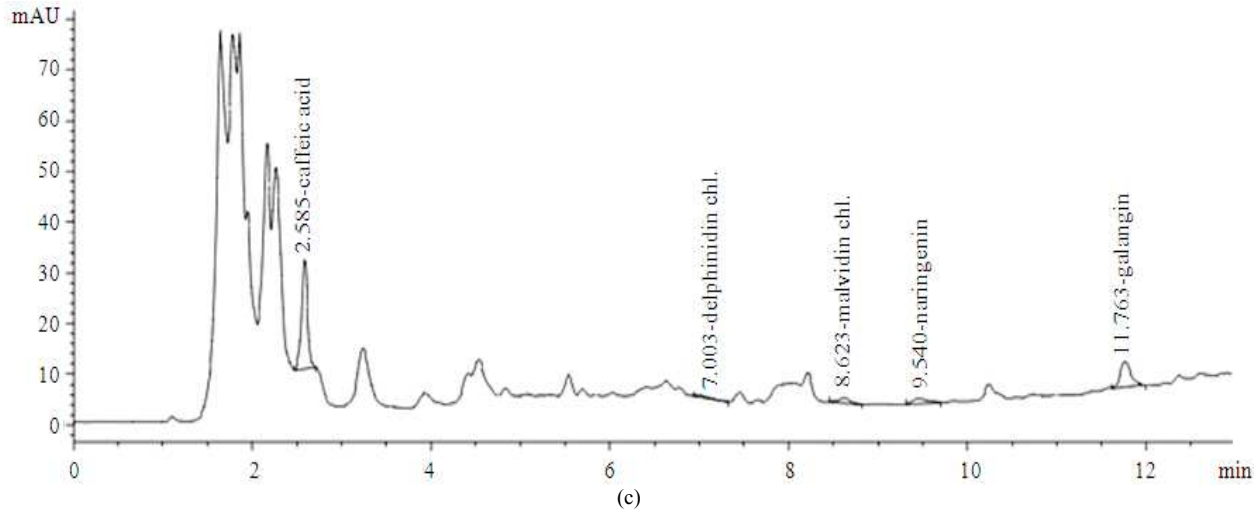
(b)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min

(c)

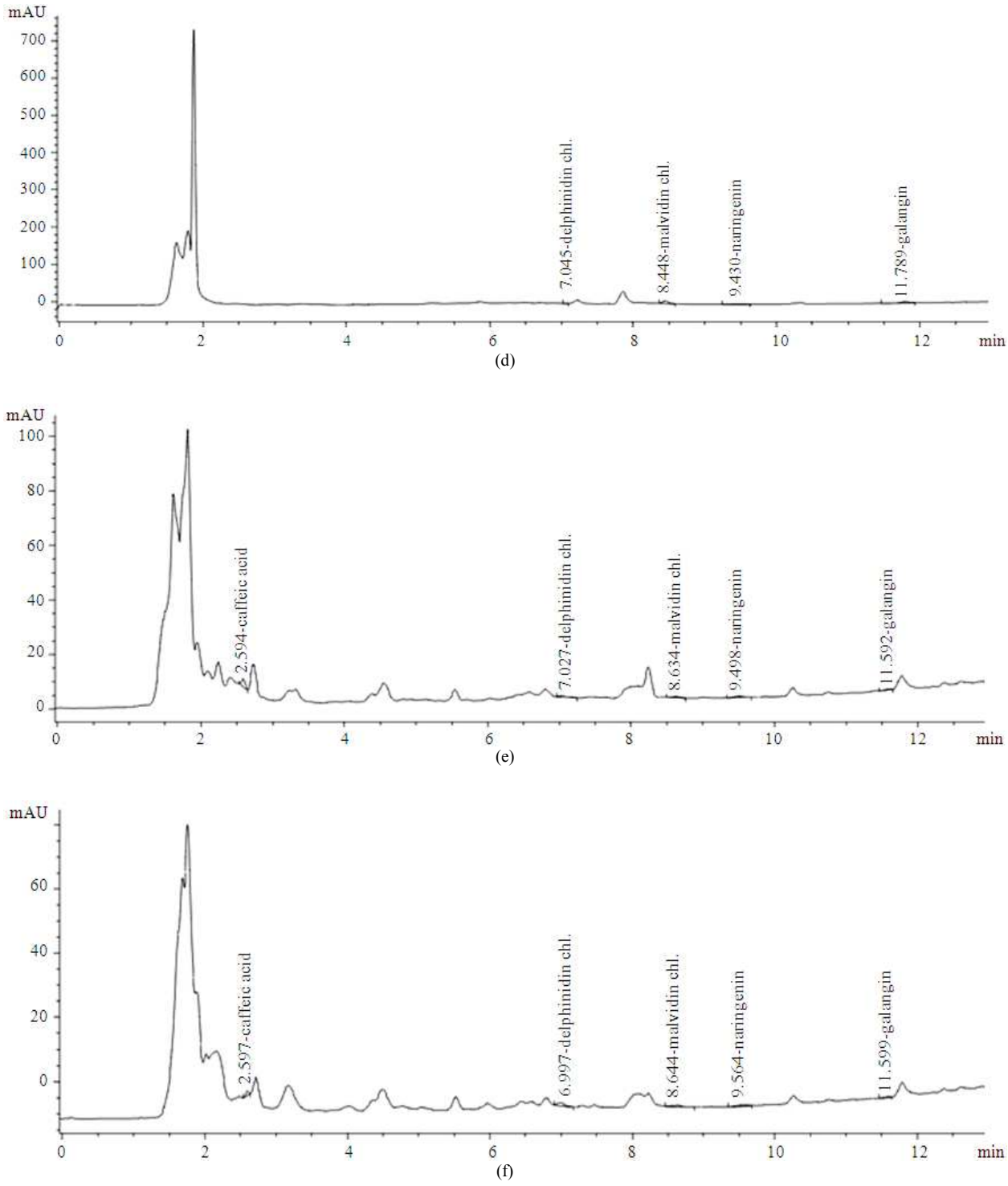

Fig. 2. HPLC analysis of (a) Caffeic acid, Delphinidine chloride, Malvidine chloride, Naringenin and Galangin standards mix, (b) Italian cultivar, (c) American cultivar, (d) Lebanese cultivar, (e) Taify$_b$ and (f) Taify$_e$

Table 3. Flavonoid contents (µg/ml) of grape (*Vitis vinifera* L.) cultivars understudy determined by HPLC method

Cultivar	Cyanidine chloride (µg/mL)	Myricetin (µg/mL)	Quercetin (µg/mL)	Chrysin (µg/mL)	Caffeic acid (µg/mL)	Delphinidine chloride (µg/mL)	Malvidine chloride (µg/mL)	Naringenin (µg/mL)	Galangin (µg/mL)	Total (µg/mL)
Italian	1.20005[a]	0.15027[a]	0.68669[a]	0.78911[a]	4.08435[a]	0[a]	1.20859[a]	2.57138[a]	1.66884[a]	12.35927[a]
American	0.65377[b]	0.39762[b]	0.30020[b]	0.54052[b]	3.12462[b]	0.30956[b]	1.66814[b]	1.11493[b]	1.42046[b]	9.52982[b]
Lebanese	1.56169[c]	0.77800[c]	0.34732[c]	0.67507[c]	0[c]	0.18919[c]	6.87797[c]	1.67817[c]	0.97748[c]	13.0849[c]
$Taify_b$	0.32025[d]	0.97915[d]	0.19127[d]	0.02208[d]	0.32722[d]	0.40882[d]	1.06506[d]	0.71181[d]	0.05742[d]	4.08308[d]
$Taify_e$	0[e]	0.15469[e]	0.10726[e]	0.02094[e]	0.12547[e]	0.77629[e]	0.98105[e]	0.45148[e]	0.04696[e]	2.66414[e]

Values within a column followed by the same letter(s) are not significantly different at the p = 0.05 level according the DMRT

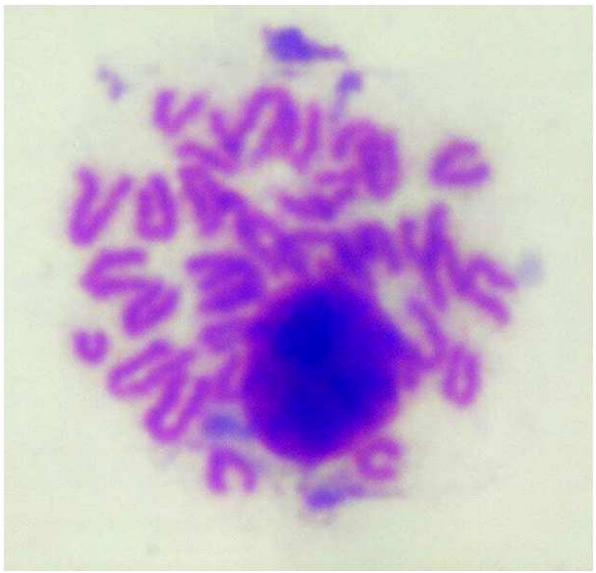

Fig. 3. Photomicrograph of mice bone marrow chromosomes after treatment of Endoxan and Lebanese grape extract showing deletion

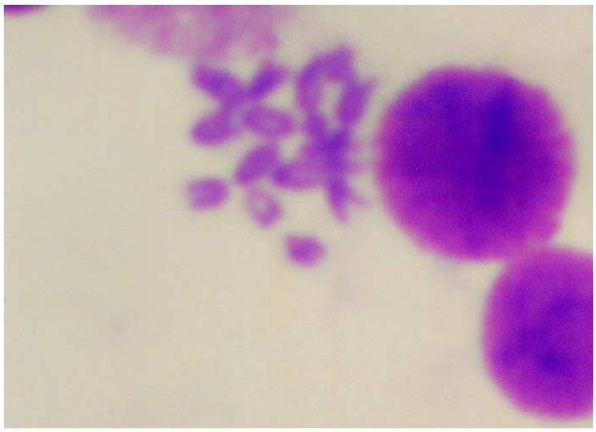

Fig. 4. Photomicrograph of mice bone marrow chromosomes after treatment of Endoxan and Lebanese grape extract showing stickiness

Table 4. Antioxidant activities of grape (*Vitis vinifera* L.) cultivars understudy expressed as DPPH free radical scavenging activity

Cultivar	Inhibition of DPPH (%)
Italian	52.75[a]
American	53.91[b]
Lebanese	40.29[c]
$Taify_b$	53.33[d]
$Taify_e$	46.96[e]

Values within a column followed by the same letter (s) are not significantly different at the p = 0.05 level according to the DMRT

Also, the anticlastogenic effect was measured as Micronulei test (MN) (**Table 6**). Using grape cultivar extracts to measure the anticlastogenic effect by micronucleus test (polychromatic erthrocytes) gave a reduction in the MN numbers. Endoxan had a high percentage of micronuclei and vitamin C reduced this effect. All grape cultivar extracts reduced the effect of Endoxan with different levels. None of grape cultivar extracts reduction effect reached the level of vitamin C reduction. Taifye had the highest reduction effect while Taifyb had the lowest effect. The same effect was noticed for anticlastogenic index.

Finally, mitotic index was measured for Endoxan and treatment with other antimutagenic compound (**Table 7**). Endoxan inhibited the mitotic index significantly. Treatments with vitamin C or with other grape cultivar extracts had a repair effect on this Endoxan inhibition effect on mitotic index. However none of them was similar to vitamin C. Taifye had the highest repair effect among cultivars.

Treatment of mice with Endoxan reduced the mitotic index (MN). Treatment with VC reconstituted MI. Treatment with grape cultivars enhanced mitotic index to levels. Therefore, the grape cultivars have the efficiency to neutralize the PC reducing effect upon the MI through its mitogenic activity. It still lower than VC.

Table 5. Averages of chromosomal aberrations in mice bone marrow after treatment with Endoxan and various grape (*Vitis vinifera* L.) extract of cultivars understudy

	Stickiness	Chromatid gap	RCF	Deletion	Total Aberrant metaphases
NC	3.51^{g}	0^{f}	0^{d}	1.2^{f}	5
PC	28.7^{a}	12.2^{a}	4.2^{a}	23.3^{a}	68
VC	9.41^{f}	3.21^{e}	0^{d}	4.63^{e}	12
Italian	13.11^{e}	4.5^{e}	1.2^{c}	8.1^{d}	31
American	16.81^{cd}	6.1^{d}	0^{d}	12.19^{c}	34
Lebanese	17.23^{c}	7.15^{c}	1.19^{c}	14.5^{c}	38
$Taify_b$	21.7^{b}	9.7^{b}	2.3^{b}	19.17^{b}	49
$Taify_e$	15.15^{de}	5.6^{d}	0^{d}	7.92^{d}	39

*; Values within a column followed by the same letter(s) are not significantly different at the p = 0.05 level according to the LSD
*; Negative Control (NC), Positive Control (PC), Vitamin C (VC), Robertsonian Centric Fusion (RCF)

Table 6. Averages of Micronuclei (MN) and Anticlastogenic Index (ACI) in mice bone marrow after treatment with Endoxan and various grape (*Vitis vinifera* L.) extract of cultivars understudy

	NC	PC	VC	Italian	American	Lebanese	$Taify_b$	$Taify_e$
MN %	0.22^{cd}	2.3^{a}	0.31^{c}	0.48^{c}	0.62^{bc}	0.86^{b}	1.1^{b}	0.45^{c}
ACI (%)	-	-	95.67	87.50	80.76	69.23	57.69	88.94

*; Values within a raw followed by the same letter(s) are not significantly different at the p = 0.05 level according to the LSD
*; Negative Control (NC), Positive Control (PC), Vitamin C (VC)

Table 7. Averages of Mitotic Index (MI) in mice bone marrow after treatment with Endoxan and various grape (*Vitis vinifera* L.) cultivars understudy

	NC	PC	VC	Italian	American	Lebanese	$Taify_b$	$Taify_e$
MI	8.51^{a}	1.43^{g}	7.5^{b}	5.19^{d}	4.81^{d}	3.76^{e}	2.6^{f}	6.2^{c}

*; Values within a raw followed by the same letter(s) are not significantly different at the p = 0.05 level according to the LSD
*; Negative Control (NC), Positive Control (PC), Vitamin C (VC)

4. DISCUSSION

Phenolic compounds are important constituents of grapes. Following sugars and acids, they are the most abundant constituents present in grapes. Phenolic compounds are a group of substances that are structurally diverse and are present in various amounts. The phenolic substances are primarily located in the seeds and skins of the berry. Therefore, a homogenization of the whole berries was used in this study. Recently, growing interests on phenolic compounds from grapes have focused on their biological activities linking to human health benefits, such as antioxidant and antimutagenic properties. At the same time, these properties were studied.

The common spectrophotometric method for the determination of the total phenolics content using the Folin-Ciocalteu reagent has been widely used in the area of viticulture. This method is based on oxidation-reduction reactions in which phenolics are oxidised and show maximum absorbance in the wavelength region between 725 and 765 nm. Significant differences were noticed among the five cultivars understudy. The difference in the content of total phenols depended on several factors such as variety, climatic and ecological factors, cultural practices and harvesting method (Klepacka *et al*., 2011). Pinheiro *et al*. (2009) determined differences in concentration of total phenolics in commercial grape juices produced from grapes belonging to Benitaka cultivar. Also, different samples of grape juice, analyzed by (Sautter *et al*., 2005), showed variations in the averages of total phenolics among cultivars. However, the wide range of differences among cultivars understudy may be due to mainly to the different genetic background of these cultivars. Other researchers confirm that the content of phenolic compounds that prevails in products made of grape may depend on relevant factors, the grape variety, the method applied for extracting the compounds and the storage conditions. According to Klepacka *et al*. (2011) the amount of phenolic compounds vary according to factors such as, climate, soil condition, grape variety, grape ripeness, grape maceration, pH and others. Grapes that are squeezed with husks, peel and seed generate larger amounts of those compounds. In a study accomplished by Baydar *et al*. (2004), on the

concentration of total phenolics in grape seeds separately and in pulp together with the grape juice, without seeds, it was verified that the seed showed higher phenolic content (647.92), whereas the husk/juice showed 37.49 mg of gallic acid extract g^{-1}.

As for the HPLC study, a complete separation of all grape phenol components by HPLC method was very difficult because phenols contain a large number of hydroxyl groups and there are many isomers. The major phenolic compounds found in grapes are either members of the diphenylpropanoids (flavonoids) or phenylpropanoids (non-flavonoids). Also Jacob *et al*. (2012) indicated that the most common naturally occurring flavonoids in grapes are cyanidine, delphinidin and malvidin. Therefore, nine important phenolic compounds (flavonoids and nonflavonoids) were chosen for analysis namely; Cyanidine chloride, Myricetin, Chrysin, Quercetin, Delphinidine chloride, Malvidine chloride, Naringenin, Galangin and Caffeic acid. Due to the overlapping of the standards, they divided into two groups. Standard mix I which consisted of Cyanidine chloride, Myrecitine, Quercetine and Chrysine and standard mix II which consisted of Caffeic acid, Delphinidine chloride, Malvidine chloride, Naringenine and Galangin. The HPLC study of nine important phenolic compounds indicated that the differences among the five cultivars for the total of nine phenolic compounds were significant. At the same time, significant differences among cultivars were obtained for each compound, while the highest cultivar for each compound differed from compound to another. These findings confirmed the different genetic background of each cultivar and the cultivars Italian and Labanese contained the highest amount of phenols.

The scavenging activity on DPPH radicals has been widely used to determine the free radical-scavenging activity of different matrices (Pereira *et al.*, 2006; Sousa *et al.*, 2008; Oliveira *et al.*, 2007; 2008). DPPH is a stable free radical that is dissolved in methanol and its purple color shows a characteristic absorption at 517 nm. Antioxidant molecules scavenge the free radical by hydrogen donation and the color from the DPPH assay solution becomes light yellow resulting in a decrease in absorbance. Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation (Hatano *et al*., 1989). In this assay, results are expressed as the ratio percentage of the absorbance decrease of DPPH radical solution in the presence of extract at 517 nm to the absorbance of DPPH radical solution at the same wavelength. The antioxidant activity of grape cultivars were estimated using the quenching intensity of DPPH. The data showed that the grape cultivars were able to quench 47-60% of DPPH.

Although, it is well established that the phenols are the main compounds that are responsible of antioxidant activity of grape (Xia *et al.*, 2010), the present result showed no correlation between the total phenols and the antioxidant activity. The relationship between phenolic compounds and antioxidant capacity was inconsistent among the results from different studies. Pereira *et al.* (2006) and Sousa *et al.* (2008) proved that antioxidant activity values were statistically correlated with total phenols content in their analyzed samples. Also, Yamauchi *et al.* (1992); showed almost equal inhibitory effects of grapes from different places of Japan, China, Brazil and USA. Banskota *et al.* (2000) obtained same results for nine different grape samples from Brazil, Peru, the Netherlands and China.

However, it is demonstrated that antioxidant activity of grape and grape-derived products are influenced, not only by their content of polyphenols, but also by their phenolic compositions, all of which are influenced by vintage, grape variety and ageing conditions (Davalos *et al*., 2005), which indicated that, besides the concentration, the antioxidant capacities of phenolic compounds were affected by other factors (Radovanovic *et al*., 2009; Majo *et al*., 2005). In a study, malvidin-3-glucoside showed the highest antioxidant capacity in grape anthocyanins (Rivero-Perez *et al*., 2008). Although total phenolic index was lower in grape flesh than in grape skin because anthocyanins were absent in the flesh, they possessed equal amounts of reactivity to hydroxyl radicals (Falchi *et al*., 2006). In another study, the results also showed that the anti-radical activity was due to the flavanols, rather than anthocyanins (Arnous *et al*., 2002). The result suggested that perhaps the antioxidant capacity of phenolics has a concentration saturation limit and above this limit, the activity could not increase further with the concentration (Dani *et al*., 2012).

The antioxidative characteristics of grape phenolic compounds are mainly ascribed to their free radical scavenging and metal chelating properties, as well as their effects on cell signaling pathways and on gene expression (Soobrattee *et al*., 2005). The mechanism was mainly speculated to react directly to generate phenoxyl radicals (Yoshimura *et al*., 2003), which was stable and cuts off the reaction chains. The chemical functional group and structure is OH for antioxidant capacity of phenolic compounds). When the OH added onto the flavonoid nucleus, the activity enhanced, while substituted by the

OCH3 groups, the activity diminished. The results were proved by (Majo *et al*., 2008). The o-diphenoxyl groups in resveratol were determined to exhibit higher antioxidant activity than other compositions (Qian *et al*., 2009).

To measure the antimutagenic activities mice was treated with grape extracts which resulted in a decrease in all types of Chromosomal Aberrations (CAs). Treatment with vitamin C along with Endoxan as potent antioxidant significantly reduced all CAs. Treatment with grape cultivars has similar effects to that of treatment with VC. Treatment with grape cultivars after the treatment with Endoxan (T1) did not eliminate the mutagenic effect of Endoxan completely as in the negative control group, but it set it back to insignificant levels of VC effect. Antimutagenic activity for some grapes phenolic components; caffeic acid, cinnamic acid, genstein and diazedien; against mutations induced with some agents such as benzo[a]pyrene, 3-amino-1,4-dimethyl-5H-pyrido[4,3b]indole (Trp-P-1) and sodium azide were reported (Irulappan and Natarajan, 2007). Grape cultivars extract caused statistically significant decrease in the frequency of chromosome damage induced by Doxorubicin (DXR) compared to the group treated only with DXR. This reduction might be, in part, due to the presence of phenolic compounds in the studied grape cultivars, which are able to remove free radicals produced by mutagenic agents such as DXR (Tzvetan *et al.*, 2007).

Using grape cultivars to measure the anticlastogenic effect by Micronucleus (MN) test (polychromatic erthrocytes) gave a reduction in the MN numbers. The MN data suggest that grape cultivars have good effect to reduce the MN averages. It seems that the grape cultivars have more powerful effect as antimutagenic effect than its efficiency as anticlastogenic effect. This might support the idea that the grape cultivars work at the cellular level to avoid the mutation damage by its antioxidant activity rather than its activity to reduce clastogenic effect that mainly are induced by cellular activities as well as direct structural mutation damage. It is documented that polyphenols have significant reduction effect on the frequency of micronucleated cells in bone marrow cells and peripheral blood cells. They are effective in preventing DNA damage and one of the mechanisms of action might involve scavenging of active oxygen radicals (Yamagishi *et al*., 2001). They showed a significant anticlastogenic activity before and after X-ray irradiation treatments. Also, they have free oxygen radicals and lipoperoxyradicals scavenging activities (Flavonoids in citrus extract of *Citrus aurantium* var. *amara* significantly reduced the clastogenic effect of radiation on mice bone marrow (Hosseinimehr *et al*., 2003). The flavonoids quercetin and its glucoside isoquercitrin reduced the number of micronuclei in polychromatic erythrocytes of the bone marrow of mice (Edenharder *et al*., 2003).

Accordingly, treatment of mice with Endoxan reduced the mitotic index (MN). Treatment with VC reconstituted MI. Treatment with grape cultivars enhanced mitotic index to levels. Therefore, the grape cultivars have the efficiency to neutralize the PC reducing effect upon the MI through its mitogenic activity. It still lower than VC. Other studies reported the similar effects of grape cultivars on mitotic index (Tzvetan *et al*., 2007).

Finally, the antigenotoxic activities of grape extracts are reasonable because it is well known that consumption of fresh fruits and vegetables is associated with decline in genotoxic incidence (Steinmetz and Potter, 1996). It is due to many phenolic active compounds which can trap the aggressive metabolites of mutagens. It is well known that many mutagens act via radical mechanisms and hence damaging biologically important molecules including DNA (Hussain *et al*., 2003). Many vegetables and fruits are known to prevent chromosomal and DNA damage in animals (Nersesyan *et al*., 2004; Miyata *et al*., 2004). Radicals, which can induce damage in biologically important molecules can be trapped by antioxidants and hence preventing genotoxicity (Steinmetz and Potter, 1996).

5. CONCLUSION

This study provides preliminary evidence that the grape extract of some cultivars from Taif region were able to exhibit potent antioxidant activity by quenching DPPH radical solution. At the same time, the present study showed no correlation between the total phenols in the extracts and the antioxidant activity. Also, the grape extracts demonstrated antimutagenic ability by decreasing chromosomal aberrations, micronuleus numbers and mitoic index inhibition in bone marrow cells of mice induced by a powerful mutagen Endoxan. The antioxidant and antimutagenic activities of grape extracts are reasonable because they contain many phenolic active compounds which can scavenge free radicals and trap the aggressive metabolites of mutagens.

6. ACKNOWLEDGEMENT

The researchers are grateful to Taif University, KSA for financial support to achieve the present research study. This study was supported by Taif University grant no. (1362-432-1). Sincere thanks are extended to all staff members of this study in Biotechnology Department, Faculty of Science for their partnership.

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