J. Food and Dairy Sciences, Mansoura Univ., Vol. 1 (5): 321 - 327, 2010

ANTICANCER ACTIVITIES OF PHENOLIC COMPOUNDS FROM RED ROOMY GRAPE FRUITS AND ITS LEAVES EXTRACTS.

Yonis, A. A. M. ; O.i.A. Elnahas and Wafaa, A. Moselhi
Home Economics Dept, Fac. of Specific Education, Mans. Univ., Egypt

ABSTRACT

A study was carried out to evaluate the anticancer activities of red roomy grape (whole fruit, seeds and leaves) extracts. The methanolic (80%) extract was evaporated to dryness, the residue (crude extract) (CE) was fractionated by using two solvents, i.e. ethyl acetate fraction (EAF) and butane fraction (BUF). EAF extract contained total phenol compounds 55.03%, 29.90% and 5.40%, while BUF extract contained 52.06%, 33.65% and 8.10% and CE contained 43.39%, 20.93% and 1.70% in whole fruit, seeds and leaves respectively. The results revealed that all extracts of red roomy grape (whole fruit, seeds and leaves) were effective as anticancer agent since their activities ranged from 70.05% to 79.91%, 54.34% to 61.22% and 18.15% to 42.50% at concentration of 10 µg/ml in whole grape sample, seeds and leaves respectively.
Keywords: Anticancer, grape, chemical constituents, phenolic compounds.

INTRODUCTION

Fruits and vegetables are known lately to strongly contribute in reducing risks of diseases of various etiologies as cancer and heart stroke. This fact is attributed to the large amounts of antioxidants they contain (Kris-Etherton *et al*., 2002 and Ha¨kkinen *et al*., 1999). Phenolic compounds are a group of biologically active molecules present as metabolites in plants. The interest for these natural compounds has increased in the last years due to their antioxidative, and anticarcinogenic activity and relationship to human health. It has been demonstrated that some health benefits of food depend on the presence of these antioxidants, which occur in fruits and vegetables in general. In this study, grapes (whole seeds and leaves) were used as sample due to the fact that a number of phenolic compounds have been detected in berries conferring those anticarcinogenic properties (Eduar do *et al*., 2003). Antioxidant compounds are produced by the plant to protect the cell against the attack from other cell chemical species as free radicals and reactive oxygen species. Free radicals are constantly produced by the cell metabolism (Benavente-Garcý´a *et al*., 1997). Phenolic compounds contain aromatic ring(s) bearing hydroxyl group(s) and can range from simple molecules to very large oligomers. They frequently occur naturally in glycosylated forms, which make them more water-soluble although the higher molecular weight oligomers are more insoluble (Bravo, 1998). Phenolic compounds are abundant in highly colored fruits, and due to their popularity and high consumption, these fruits serve as one of our most important dietary sources of phenolics (Williner, *et al*., 2003). In fact, polyphenolic compounds of plants are well known as exhibit contrasting pharmacological actions, such as prooxidant activity and the induction of cell death (Lapidot, *et al*., 2002). In

this regard, cell culture is a powerful technique for studying physiological, biochemical and toxicological processes modulated by pure phytochemicals in vitro (Glei *et al*., 2003). (HEPG2) cell line Liver carcinoma has been widely used in biochemical and nutritional studies because it is considered one of the experimental models that more closely resembles the human hepatocyte in culture (Ramos, *et al*., 2005). In addition, steady-state functioning of the antioxidant defenses in HEPG2 is relatively higher than in hepatocytes and other non-transformed cells. Therefore, variations in the responses to different conditions are more easily detected (Alia, *et al*., 2005).

The present works takes into consideration the use of red roomy grape (whole fruit, seeds and leaves) extracts as source of phenolic compounds to evaluate their activities as anticancer agents. Also, separation on some chemical components of different fractions (ethyl acetate fraction and butane fraction) from methanolic extracts and its effects as anticancer agent were studied.

MATERIALS AND METHODS

Materials:

Fresh grape (*Vitis vinifera*) fruits and its leaves were obtained from the Agricultural Research Center, Giza Egypt.

Extractions:

- Methanolic extract (crude extract CE): Fresh grape fruits were washed and freeze dried immediately while, seeds and leaves were dried at 60º C. Then the dry materials were ground, the powdered were macerated in methanol 80% (1:3 w/v) for 24h. The methanolic extracts were filtered and evaporated under vacuum up to dryness; the residue was named crude extract (CE).
- The crude extracts (CE) were dissolved in distilled water and then partitioned with ethyl acetate (6 times x 200). The ethyl acetate layers dehydrated with Na2SO4 and evaporated to dryness. The residue were named ethyl acetate fraction (EAF). The remaining water layer then was partitioned with n – butanol (6 times x 200ml). The butanol layers dehydrated with Na2 SO4 were evaporated to dryness. The residues were named butanol fraction (BUF).

Chemical analysis:

– Moisture, protein, fat, ash, fiber, minerals and vitamins contents were determined using the methods described by A.O.A.C. (2005).

-Total phenol content was determind by colorimetric method of Shahidi and Naezk (1995).

HPLC analysis:

The samples were performed in National Research Center Cairo by HPLC (HP) equipped with a Hewlett- Packard 1050 photodiode array detector (Agilent Technologies Palo Alto. Calif. U.S.A.) with Hewlett – packared. HPLC, Chem. Station software and auto sampler. Using a PDS- column C 18 – 5 micron (150mm x4-6mm, operated at 45º C. The solvent system used was gradient of a (acetic 2.5%) B (acetic 8%) and C (acetonitrile). The

Solvent flow rate was 1 ml/min. Ingection volume 50 µl. Phenolic compounds were assayed by external standard calibration at 280 nm.

Anticancer activity (cytotoxicity activity) against tumor cell lines (HEPG2):

Cytotoxicity was determined in National Cancer Institute. Cairo Univ, according to the method described by Skehan and Streng (1990).

RESULTS AND DISCUSSION

The chemical composition of red roomy grape (whole fruits, seeds and leaves) are given in Table (1). The results of the analyses were established to give nutrient values per 100 g. Moisture, protein, fat,ash,carbohydrate and fiber were found 78.3%, 0.62%, 0.68%, 0.59%, 18.71% and 1.1% in whole grape fruit respectively, while, 6.93%, 7.39%, 17.99%, 2.43%, 16.36% and 48.9% in seeds respectively, and 73.6%, 5.1%, 2.3%, 2.1% 5.70% and 11.2% in leaves respectively. Also, mineral contents, vitamins A&C and total phenolic compounds are presented in the same table. According to results, calcium (Ca), iron (Fe), magnesium (Mg), phosphorus (P), potassium (K), vitamin A, vitamin C and total phenolic compounds were 10.0mg, 0.37mg, 8.00mg, 24mg, 195.00mg, 82 IU, 11mg and 11.65mg in whole grape fruit respectively, while, the value were 75.1mg, 12.5mg, 25.1mg, 6.5mg, 8.3mg, 8.1 IU,23.4mg and 73.59mg in seeds respectively, and 316.0mg, 1.65mg, 58.7mg, 86.1mg, 254mg,1132.0 IU, 9.8mg and 15.73 in leaves respectively. These results are in agreement with those obtained by Sanchez et al., (2007), Hallabo et al., (2008) and Spanghero et al., (2009).

Table (1): Chemical composition of whole grape fruits, seeds and leaves of red roomy grape.

Samples / Components	Red Roomy Grape		
	whole	seeds	leaves
Moisture %	78.30	6.93	73.60
Protein %	00.62	7.39	05.10
Fat %	00.68	17.99	02.30
Ash %	00.59	2.43	02.10
Carbohydrate %	18.71	16.36	5.70
Fiber %	01.10	48.90	11.20
Calcium, Ca (mg/100g)	10.00	75.10	316.00
Iron, Fe (mg/100g)	00.37	12.50	01.65
Magnesium, Mg (mg/100g)	8.00	25.10	58.70
Phosphorus, P (mg/100g)	24.00	6.50	86.10
Potassium, K (mg/100g)	195.00	8.30	254.00
Vit. A (IU/100g)	82.00	8.10	1132.00
Vit. C (mg/100g)	11.00	23.40	9.80
Total phenolic compounds (mg/100g)	11.65	73.59	15.73

Data in Table (2) indicated that red roomy grape (whole fruits, seeds and leaves) contained 11 phenolic components (ppm/100g) which could be identified as follows: protocachoic 8729.55, catachol 5528.42, chlorogenic 4037.23, gallic acid 860.16, catechein 759.57, synergic 440.3, pyrogallol 55.9, ferulic 13.0, coumarin11.92, caffeic 6.2 and vanillic acid 1.3 in whole

red roomy grape fruit, while, the value of chlorogenic 3901.13, gallic acid 2729.05, catechein 1789.25, coumarin 216.27, protocachoic 210.0, pyrogallol 43.2, ferulic 32.3, catachol 30.9, vanillic acid 18.9, synergic 18.4 and caffeic 4.6 in seeds, and . Protocachoic 798.47, chlorogenic 198.3, pyrogallol 132.9, ferulic 55.9, gallic acid 42.87, catechein 40.08, catachol 32.9, synergic 16.8, caffeic 5.9, coumarin 2.16 and vanillic acid 0.82 in leaves. These results are in agreement with ranges reported by Andrew et al., (2010).

Table (2): Identification of phenolic compound (ppm/100g) in whole fruits seeds and leaves of red roomy grapes.

Grape variety / Chemical constituents	Red Roomy Grape		
	whole	Seeds	leaves
Vanillic acid	1.30	18.90	0.82
Catechein	759.57	1789.25	40.08
Protocachoic	8729.55	210.00	798.47
Coumarin	11.92	216.27	2.16
Gallic acid	860.16	2729.05	42.87
Ferulic	13.00	32.30	55.90
Catachol	5528.42	30.90	32.90
Chlorogenic	4037.23	3901.13	198.30
Synergic	440.30	18.40	16.80
Caffeic	6.20	4.60	5.90
Pyrogallol	55.90	43.20	132.90

Targeted analyses of red roomy grape (whole fruits, seeds and leaves) methanol extract (CF), ethyl acetate fraction (EAF) and butanol fraction (BUF) detected total phenols are content presented in Table3. The exhibited data indicted that whole grape fruits extracts contained the higher content of total phenols EAF (55.03%) followed by BUF extract (29.9%), and CE extract (5.4%).

Table (3): Total phenol content (%) in whole, seeds and leaves red roomy grape extracts.

red roomy grape	Extracts		
	CE	EAF	BUF
Whole	43.39	55.03	52.06
seeds	20.93	29.90	33.65
leaves	01.70	05.40	08.10

CE =crude extract, EAF=ethyl acetate fraction, BUF= butanol fraction.

Regarding total phenols contet in red roomy seeds, EAF extract contained (52.06%) followed by BUF extract (33.65%), then CE extract (8.1%). Also, the results showed that EAF contained relatively higher total phenols content (43.39%) followed by BUF extract (20.93%) whereas; CE contained the lowest total phenols content (1.7%) in leaves. These results are in agreement with those obtained by Hulya, (2007) and Changmou et al., (2010).

Data recorded in tables (4), (5) and (6) indicated the anticancer activity of the fractions of red roomy grape (whole fruits, sees and leaves) extracts. Regarding crude extract (CE) 80% methanolic extract, the

anticancer activity increased from 49.92% to 70.05%, 21.56% to 54.34% and 2.19% to 18.15% in whole fruits, seeds and leaves with increasing the concentration from 1 to 10 µg/ml respectively. In the case of ethyl acetate fraction (EAF), the anticancer activity increased from 52.79% to 79.91%,21.41% to 61.22% and 3.14% to 24.50% with increasing the concentration from 1 to 10 µg/ml in whole, seeds and leaves respectively. Concerning butanol fraction (BUF), there were gradual increases in anticancer activity from 51.81% to 79.75%, 28.77% to 59.31% and 7.35% to35.10 with increasing the concentration from 1 to 10 µg/ml in whole, seeds and leaves respectively.

Generally, obtained results indicated that the anticancer activity of above mentioned fractions increased with increasing concentrations. Duthic *et al*., (2000) and Nijveldt *et al*., (2001) revealed that flavonoids as antioxidants can inhibit carcinogenesis and the antioxidant potential may be anticarcinogenic.

Table (4): anticancer activity (%) of whole red roomy grape extracts on HEPGR2.

Extracts	Concentration µg/ml.			
	1	2.5	5	10
CE	49.92	63.48	69.90	70.05
EAF	52.79	73.01	77.99	79.91
BUF	51.81	70.07	75.77	79.75

CE =crude extract, EAF=ethyl acetate fraction, BUF= butanol fraction.

Table (5): anticancer activity (%) of red roomy grape seeds extracts on HEPGR2.

Extracts	Concentration µg/ml.			
	1	2.5	5	10
CE	21.56	30.55	40.62	54.34
EAF	21.41	33.06	40.75	61.22
BUF	28.77	33.48	36.40	59.31

CE =crude extract, EAF=ethyl acetate fraction, BUF= butanol fraction.

Table (6): anticancer activity (%) of red roomy grape leaves extracts on HEPGR2.

Extracts	Concentration µg/ml.			
	1	2.5	5	10
CE	2.19	4.91	10,93	18.15
EAF	3.41	18.14	28.90	42.50
BUF	7.53	15.30	23	35.10

CE =crude extract, EAF=ethyl acetate fraction, BUF= butanol fraction.

REFERENCES

Alia, M., Ramos, S., Mateos, R., Bravo, L., and Goya, L. (2005): Response of the antioxidant defense system to tert-butyl hydroperoxide and hydrogen peroxide in a human hepatoma cell line (HepG2). Journal of Biochemical and Molecular Toxicology, 19, 119.

Andrew, P. Breksa , I., Gary, R., Takeoka, Marlene, B., Hidalgo, A., Vilches, J., Vasse, David, W. and Ramming (2010): Antioxdant activity and phenolic content of 16 raisin grape (Vitis vinifera L.) cultivaes and selections.Food chem.,121: 740.

AOAC. (2005): Association of Official Analytical Chemists. Official Methods of Analysis of the Association Analytical Chemists (18th ed.). Washington, DC: AOAC.

Benavente-Garcý´a, O., Castillo, J., Marý´n, F.R., Ortun.o, A., and Del Rý´o, J.A., (1997): Uses and Propierties of Citrus Flavonoids. J. Agric. Food Chem. , 45 (12): 4505.

Bravo, L. (1998): Polyphenols: Chemistry, dietary sources, metabolism and nutritional significance. Nutrition Reviews, 56, 317.

Changmou, X., Yali Z.,Lei C., and Jiang L. (2010): Phenolic compounds and antioxidant properties of different grape cultivars grown in China. Food chem.,119: 1557.

Duthic, G.G., Duthic, S.J., and Kyle,J.A.M. (2000): Plant polyphenols in cancer and heart disease implications as nutritional antioxidants. Nutrition Research Reviews, 13: 79.

Edur do E. P.B., Casimir C., Subramani S.,and Gerard, K. (2003): Phenolic Content and Antioxidant Capacity of Muscadine Grapes. J. Agric. Food Chem., 51: 5497.

Glei, M., Matuschek, M., Steiner, C., Bo1hm, V., Persin, C., and Pool-Zobel, B.L. (2003): Initial in vitro toxicity testing of functional foods rich in catechins and anthocyanins in human cells. Toxicology in Vitro, 17: 723.

Ha¨kkinen, S., Heinonen, M., Ka¨renlampi, S., Mykka¨nen, H., Ruuskanen, J., and To¨rro¨nen, R., (1999): Screening of selected flavonoids and phenolic acids in 19 berries. Food Res. Int. 32, 345.

Hallabo, S. A. A., Awatif, I., Ismael., Rayda, Y. M. and Marwa, E. Mohamed. (2008): Technological and biological studies on grape seed. J. Agric Sci Mansoura Univ., 33: 3483.

Hulya O., H. (2007): Total antioxidant activities, phenolics, anthocyanins, polyphenoloxidase activities of selected red grape cultivars and thier correlations. Scientia Horticulturae 111: 235.

Kris-Etherton, P.M., Hecker, K.D., Bonanome, A., Cobal, S.M., Binkoski, A.E., and Hilper, K.F., Griel, A.E., Etherton, T.D., (2002): Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am. J. Med., 113 (9): 71.

Lapidot, T., Walker, M. D., and Kanner, J. (2002): Antioxidant and prooxidant effects of phenolics on pancreatic beta-cells in vitro. Journal of Agricultural and Food Chemistry, 50: 7220.

Nijveldt, R.I., Nood, D.E., Hoorn, P.G., Boelens, K.V., and Leewen, P.A. (2001): Flavonoids: a review of probable mechanisms of action and potential applications. Am. J. Clin. Nutr., 74:418.

Ramos, S., Alia, M., Bravo, L., and Goya, L. (2005): Comparative effects of food-derived polyphenols on the viability and apoptosis of a human hepatoma cell line (HepG2). Journal of Agricultural and Food Chemistry, 53: 1271.

Sanchez-Alonso, I., Jimenez-Escrig, A., Saura-Calixto, F. and Borderias, A. J. (2007): Effect of grape antioxidant dietary fiber on the prevention of lipid oxidation in minced fish: Evaluation by different methodologies. Food Chem. 101:372.

Shahidi, F., and Naczk, M. (1995): Methods of analysis and quantification of phenolic compounds. Food phenolic, sources, chemistry, effects and applications. Technomic Published Company. Inc., Lancaster. PA. 287.

Skehan, P., and Streng, R. (1990): New coloremetric cytotoxicity assay for anticancer drug screening. J. Natl. Cancer inst., 83.

Spanghero, M., Salem, A. Z. M. And Robinson, P. H. (2009): Chemical composition including seondary metabolites, and rumen fermentability of seeds and pulp of Californian (USA) and Italian grape pomaces. Animal Feed sci. and Technology, 152: 243.

Williner, M. R., Pirovani, M. E., and Guemes, D. R. (2003): Ellagic acid content in strawberries of different cultivars and ripening stages. Journal of the Science of Food and Agriculture, 83: 842. 1107.

الأنشطة المضادة للسرطان للمركبات الفينولية المستخلصة من ثمار العنب الرومى الأحمر و أوراقه

عـلاء الـدين احمـد مرسـى يـونس ، أسـامه ابـراهيم عبـدالرءوف النحـاس و وفاء عبدالهادى مصيلحى

كلية التربية النوعية- قسم الأقتصاد المنزلى – جامعة المنصورة

أجريت هذه الدراسة لتقييم نشاط العنب الرومى الأحمر(الحبة كاملة و البذور و الأوراق) كمضاد للنشاط السرطاني . حيث تم عمل مستخلص ميثانولي (٨٠%) ثم تبخير الميثانول منه و أخذ الجزء المتبقي من المستخلص الخام (CE) و تجزئته باستخدام المذيبان ايثيل أستات والبيوتانول و الحصول علي جزء الايثيل استيات وجزء البيوتانول بالإضافة للمستخلص الخام .

و أسفرت النتائج أن المستخلص الخام يحتوي على ٤٣,٣٩% و ٢٠,٩٣% و ١,٧% من الفينولات تحتوي الحبة الكاملة و البذور و الأوراق على التوالي. بينما احتوي المستخلص الايثانولي من الفينولات علي ٥٥,٠٣% و ٢٩,٩% و ٥,٤% و مستخلص البيوتانول علي ٥٢,٠٦% و ٣٣,٦٥% و ٨,١ % من الفينولات في الحبة الكاملة و البذور و الأوراق علي التوالي.

و نستخلص من النتائج المتحصل عليها أن كل من مستخلصات العنب كان لها أكبر الفاعلية كعوامل مضادة للنشاط السرطاني حيث يتراوح نشاطها من ٧٠,٠٥% إلي ٧٩,٩١% فى الحبة الكاملة و ٥٤,٣٤% الى ٦١,٢٢% فى مستخلصات البذور و ١٨,١٥% الى ٤٢,٥% فى مستخلصات الأوراق و ذلك عند تركيز ١٠ ميكروجرام/ مل.

قام بتحكيم البحث

أ.د / عبد الحميد ابراهيم عبد الجواد — كلية الزراعة – جامعة المنصورة

أ.د / عبد الغنى محمود عبد الغنى — كلية التربيه النوعيه – جامعة المنصوره