Vinification Wastes: Polyhenolic profile and anti-cancer activities assessment of grape stem extracts

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The wineries' wastes are harmful for the environment due to their high organic load and high acidity attributing mainly to their polyphenolic content. For this reason, most of the wine-producing countries as well as other institutions (e.g. EuropeanUnion) have established regulations for the proper management of the winery's waste. A major part (3–5% of the processed grapes)of the solid wastes of wineries is composed of grape stems (Mullin,2011). Currently, a small portion of grape stems is used as animal feed and/or in the production of natural organic fertilizers (compost), processes of limited economical interest, while the majority, along with other wastes of the wine making process, is discarded in nearby open fields.

The environmental problems faced introduce the need for the development of method for their proper management via utilization. Interestingly, one of our previous studies has shown that grape stem extracts are rich in bioactive polyphenols such as flavonoids, stilbenes and phenolic acids, suggesting their potential use as a source of high added value polyphenols, an activity that would combine the profitable venture with the environmental protection in wine-producing zones. Moreover, we have recently shown that grape stem extracts display significant antioxidant activity, protective activity against ROS-induced DNA damage and inhibitory activity against human hepatocellular and cervical cancer cells (Apostolou et al., 2014).Having in mind the well established anticancer properties of grape stem extracts against liver and cervical cancer cells, this endeavor aims to extend these studies in order to investigate the inhibitory effects of the extracts against the growth of additional human cancer cell types such as breast, renal, thyroid and colon.

In addition to the polyphenolic composition, the presence of various flavanols, flavonols, phenolic acids and stilbenes was determined (Table 1). In particular, gallic (from 8.38 to 42.29 mg/gdried extract) and syringic acids (from 0.80 to 32.23 mg/g dried extract) were found to constitute the most abundant phenolic acids, while (+)-catechin (from 7.35 to 12.18 mg/g dried extract) and(−)-epicatechin (from 4.51 to 19.13 mg/g dried extract) were the prevailing flavanols. Among flavonols, the molecules of quercetin (from 3.94 to 10.24 mg/g dried extract) and rutin (from 4.47 to41.83 mg/g dried extract) were the most abundant, while the most intriguing finding was the large amounts of trans-resveratrol, a naturally occurring stilbene that is produced by a wide variety of plants in response to stress, injury, ultraviolet (UV) irradiation and fungal (e.g. Botrytis cinerea) infection.

During the last decade there are numerous research results suggesting that, besides the antioxidant activity, trans-resveratrol displays potent anticancer (chemopreventive and therapeutic) activities (Aggarwal et al.,2004). The latter is associated with the ability of the molecule to suppress the proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers, multiple myeloma, breast, prostate, stomach, colon, pancreas and thyroid cancers, melanoma, head and neck squamous cell carcinoma and ovarian and cervical carcinoma(Liu et al., 2007).

Table 1. Polyphenolic composition of grape stem extracts from different Greek *Vitis vinifera* varieties.

Grape variety	White variety	Red varieties		
	Assyrtiko	Mavrotragano	Voidomato	Moshato[d]
	2011[b]	2011[b]	2011[b]	2009[b]
Gallic acid	8.38	11.48	42.29	14.82
(+)-catechin	7.35	12.18	9.10	9.33
(-)epicatechin	15.23	19.13	4.51	13.32
trans-resveratrol	2.15	9.09	25.41	15.32
Quercetin	7.54	3.94	10.24	8.21
Kaempferol	1.04	0.74	4.08	0.67
Caffeic acid	0.31	0.54	1.78	0.58
Syringic acid	0.80	17.44	2.85	32.23
Coumaric acid	0.41	0.75	1.55	0.93
Ferulic acid	0.31	3.59	0.59	0.51
Rutin	16.00	15.93	4.47	41.83
TPC[a]	372	415	318	407

[a]TPC: Total polyphenolic content as mg gallic acid / g dried extract. [b]Harvest year. [c]All values are mg/g dried extract.

Human cancer cell lines used as targets were MCF-7 (breast, hormone dependent, ER positive), MDA-MB-231 (breast, hormone independent, ER negative), Caki-1(kidney carcinoma), 786-O (renal adenocarcinoma), K1 (thyroid carcinoma) andHT29 (colon). Cell survival was estimated after 72 h of exposure to increasing concentrations of the tested extracts by means of the SRB assay. Taking into account that the tested agents were plant extracts, the in vitro cytotoxic activities of the extracts seem very promising. Our results revealed that all administered extracts caused a dose-dependent inhibition of cell proliferation. A 50% growth-inhibitory effect (IC50) was calculated for all extracts and the respective values are shown in Table 2.

Table 2. Cytotoxicity evaluation of the administered extracts expressed in IC_{50} values as determined based on SRB assay.

Extracts	**IC_{50} values (μg/ml)***						
	Cell lines						
	MCF-7	**MDA-MB-2**	**HT29**	**K1**	**HeLa**	**786-O**	**Caki-1**
Voidomato	121	120.5	175	159	60	200	134
Mavrotragano	230	124 (109.98)	260	300	101	225	> 400
Assyrtiko	180	184	309	314	100	225	100 (104.14)
Moshato Hambourg	154 (153.57)	166	236	171	125	180	> 400

*IC_{50}

values were derived from the corresponding dose-effect curves drawn from sextuplicate determinations with CV lower than 5% (in parentheses are indicated the IC_{50} values derived from the corresponding trend lines).

This indicated that all extracts inhibited cell proliferation, with IC_{50} values 121–230 μg/ml (MCF-7),121–184 μg/ml (MDA-MD-23), 175–309 μg/ml (HT29), 159–314 μg/ml (K1), 180–225 μg/ml (786-0) and134–>400 μg/ml (Caki-1). In conclusion, the aforementioned results reveal for the first time the anti-carcinogenic potentials of grape stems extracts against colon, breast, renal and thyroid cancers. Additionally, the ability of grape extracts to inhibit the growth of renal and thyroid cancer cells was also highlighted. Thus, the present results suggest that grape stem extracts may be beneficial for human health, since they may be used for prevention or even treatment against different cancer types. Moreover, grape stems constitute a major by product of the wine making process and their determined herein significant chemopreventive properties are indicative of their value as raw materials for the development of food supplements or ingredients for the preparation of high added value functional foods. In this respect further research is necessary for the determination of the molecular mechanisms involved in their anticancer activity as well as for the assessment of their cytotoxicity in normal cells.

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