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HPLC/DAD/TOF Characterization of Selected Red Wine Solid Fractions and in *Vitro Test* on a Model of Colorectal Cancer Cells

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Summary: *HPLC/DAD/MS analysis led to the selection of two samples of Vitis vinefera L.: a free-run wine power extract, particularly rich in anthocyanins, and a deposit enriched in flavonols. These different extracts were in vitro tested on human colon cancer cell line HCT8-β8 and both have showed an anti-proliferative activity.*

Keywords: *functional food, food quality, health effect*

Introduction

Colorectal cancer (CRC) is one of the most common malignances and a leading cause of cancer deaths for both men and women. It has been observed that high levels of expression of ERβ characterized the healthy colonic mucosae, while these levels decrease with the progression of CRC. This has been suggested the idea that the ERβ could have an effect as tumor suppressor and that could be also responsible for the protective effect of estradiol against CRC [1]. Phytoestrogens are non-steroidal polyphenolic compounds (lignans, coumestans, isoflavonoids and flavonoids) which are naturally present in plants and structurally and functionally able to act as human estrogen-agonists, thanks to the higher binding affinity to ERβ. Several studies have demonstrated that phytoestrogens have a preventive effect against several type of cancer [2]. For this reason, these polyphenolic compounds seem to be ideal candidates for the prevention of CRC [3-4]. Flavonoids, which are founded in almost all fruits and vegetables, are the most abundant phytoestrogens in human diet. Among them the quercetin is one of the flavonoid which is abundant in tea, in many types of fruit and vegetables. Several evidences have shown the protective effects of quercetin against cardiovascular diseases and against cancer progression. Since the quercetin has been resulted to be the flavonoid most present in red wine, we have decided to chemically analyzed, by HPLC/DAD/TOF analysis, different extracts from red wine during the production chain, selecting, after, the samples of red wine extracts which contain quercetin derivatives. After that we have *in vitro* tested the effects of these different extracts on the human colon cancer cell line HCT8-β8 engineered to overexpress ERβ [5].

Experimental

Chemical characterization of extracts was performed by HPLC/DAD/MS techniques. Analyses were carried out using an HP 1100L liquid chromatograph equipped with a DAD detector (Agilent Corp, Santa Clara, CA, USA). Compounds were separated by using a Luna RP18 250 x4.60 mm, 5µm (Phenomenex). The HPLC system was interfaced with an Agilent TOF MS equipped with an ESI source. The TOF/MS analysis worked using full-scan mode and the mass range was set to m/z 100–1500 in both positive and negative modes. The acquisition and data analysis were controlled using Agilent LC-MS TOF Software (Agilent, USA). The HPLC-DAD quantitative analysis of each compound has been performed using specific calibration curves, build with reference standards.
The human colon cancer HCT8 cell line was obtained from the American Type Culture Collection (Rockvylle, MD, USA). Cells overexpressing human ERβ (HCT8-β8) were established as reported were established by a stable transfection with the mammalian expression vector pCXN2-hERβ [5]. 17β-estradiol (17β-E2) (internal positive control), Quercetin and Fulvestrant (ICI 182, 780) were purchased by Sigma-Aldrich (St. Louis, MO; United States). The effects of the red wine extract (1, 2.5, 5, 10, 25, 50 µM), of Quercetin (5, 10, 25, 50 µM) and 17β-E2 (10 nM), as control, were evaluated on the HCT8-β8 cell line. The effects of quercetin and 17β-E2 were assessed in presence of Fulvestrant (1µM), too.

Results

HPLC/DAD/MS analysis led to the selection of two samples of *Vitis vinefera* L.: a free-run wine power extract (extract 1), particularly rich in polyphenolic substances, and a deposit found in wine enriched in flavonols (extract 2), in particular in quercetin aglycone, a phytoestrogen with known anti-proliferative mechanisms.
The free-run wine extract is a red colored powder with an high content of anthocyanins (79%) (Table 1). The flavonols, mainly constituted by quercetin derivatives, are the 4.3%. The powder was dissolved in 5% of acidified water and analyzed by HPLC/DAD/MS.

Table 6. free-run wine power extract (extract 1)

	mg/g
procyanidins	81.0
flavonols	32.7
Caffeic acid derivatives	46.0
Anthocyanins	601.0

Quercetin glycosides are extracted from the grape skins during fermentation. The quercetin glycosides then hydrolyze in the acidic wine conditions to release the free quercetin. The quercetin may then crystallize, incorporating molecules in the process and form a deposit. The wine deposit selected was washed or extracted with $EtOH/H_2O$ and led to a red color deposit that was subdivided in three portion extracted separately with acetonitrile, acetone and methanol and analyzed in HPLC/DAD/MS. The quasi-molecular ion of quercetin 301 $[M-H]^-$ and dimer 603 $[2M-H]^-$ (confirming the quasi-molecular ion) was found in all the extracts (figure 1). The MS analysis also confirmed the presence of quercetin glucoside and quercetin glucuronide.

The results obtained on the HCT8-β8 cell line have showed an anti-proliferative activity of the free–run wine power extract (extract 1) and also of the deposit rich in quercetin aglycone (extract 2), as observed with the 17β-E2 and the Quercetin. Moreover we have preliminary observed that the anti-proliferative activity of the extract 2 (5μM of polyphenolic compounds) is higher (677%) than that (265%) showed by the extract 1 at the same concentration.

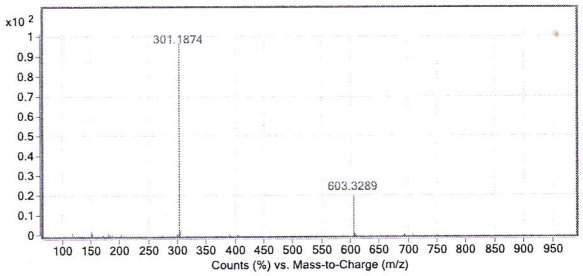

Figure 1. MS spectrum of Quercetin in extract 2

Conclusions

In this study we have selected two solid fractions derived from *Vitis vinifera* L. by HPLC/DAD/MS analysis, and one of these is enriched in quercetin aglycone.

The preliminary results obtained by the *in vitro* studies on the effects of these extracts on the biology of this CRC cellular model have shown that both these red wine extracts, in particular the extract rich in quercetin aglycone, have an anti-proliferative effect on our *in vitro* model of CRC. Nowadays, the studies on the evaluation of the correlation between the anti-proliferative effect observed, the interaction with the ERβ and the expression levels of ERβ are in progress in our laboratories.

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