Valorization of Vegetable Waste: Identification of Bioactive Compounds and Their Chemo-Enzymatic Optimization

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Abstract: A growing demand – above all in industrialised countries – for safer foods and cleaner production processes has been observed in recent times. This has led to a drive, by the agro-alimentary industries, for waste reduction and upgrading as a strategy to reduce costs and achieve new sources of income. In fact, waste may be a source of high-added value products potentially useful as beneficial food constituents, food flavours and antioxidants, cosmetics, chemopreventive agents, drugs or drug adjuvants. In this regard, we have recently examined some vegetable wastes available in the Mediterranean basin as bioresources of useful compounds and we report here a short account of our work as an example of integrated approach between the query of agro-food industry for waste upgrading and the search of some sectors of chemical industry for bioactive products obtainable from renewable resources. We employed two different strategies aimed to the exploitation of vegetable by-products: 1) Identification or isolation of bioactive natural products in vegetable waste as possible source of lead compounds or enriched fractions. 2) Chemical and enzymatic modification of lead compounds available from vegetable waste to obtain optimized analogues, food additives, drugs or cosmetics. The results here summarised are focused on: a) isolation of antiproliferative constituents from almond (*Prunus dulcis*) hulls and grape (*Vitis vinifera*) stems; b) chemical analysis of extracts / fractions with antioxidant properties obtained from grape pomace and grape stems; c) chemical / enzymatic modification of hydroxytyrosol (**26**) and *trans*-resveratrol (**11**), two bioactive polyphenols available from vegetable waste.

Keywords: Antioxidant activity, antiproliferative activity, chemo-enzymatic reactions, natural products, polyphenols, *Prunus dulcis*, terpenoids, vegetable waste, *Vitis vinifera*.

1. INTRODUCTION

At the moment the agro-industry is undergoing new pressures from the new demands of the market and society in general: indeed, there is a greater attention to the quality of life and sustainable development. One may therefore see a growing demand – above all in more industrialised countries – for safer foods and beverages and at the same time for cleaner production processes. This has led to a drive, by the industries of the agro-alimentary sector, for waste reduction and upgrading as a strategy to reduce costs and achieve new sources of income, with a positive impact on public opinion. Large amounts of vegetable waste are accumulated worldwide: millions of metric tons of various pomace waste originate from viticulture, olive oil production, tomato processing, citrus processing, as well as from sugar or cassava production. [1]. The fate of vegetable waste has been, up to recent times, mainly addressed to discharge. This is particularly true in South Europe, an area rich in agro-industrial production, with special reference to some fruit crops, like citrus, grape, olive and almond. In the last decade, however, the increasing attention to the prevention of environmental pollution, as well as to the rationalization of the agro-industrial cycle has stimulated the search for a possible exploitation of residual vegetables. The use of vegetable waste for animal feed without pre-treatments is complicated by animal intolerance to some waste components. Bioconversion or distillation, this latter recovering only volatile constituents, are applied only in some areas or for some by-products. Even maceration for composting or incineration may cause problems due to the known germination inhibition properties of many polyphenols [2], the presence of other bioactive substances [3], as well as the formation of 'off-odours'. Thus, there is a growing interest in recycling of biomasses of agro-industrial origin through extraction and upgrading. In fact, waste may be a source of high-added value products potentially useful as beneficial food constituents, food flavours and antioxidants, cosmetics, chemopreventive agents, drugs or drug adjuvants. As a continuation of our work aimed to isolation and structure elucidation of bioactive natural products [4], we have recently examined some vegetable wastes largely available in the Mediterranean basin as bioresources of useful compounds. Our work is based on two different strategies aimed to the exploitation of vegetable by-products: 1) Identification and/or isolation of bioactive natural products in vegetable waste as possible source of lead compounds or enriched fractions. 2) Chemical and/or enzymatic modification of lead compounds available from vegetable waste to obtain optimized analogues, food additives, drugs or cosmetics. Thus, we wish report here a summary of our recent work as an example of integrated approach between the query for waste upgrading of agro-food

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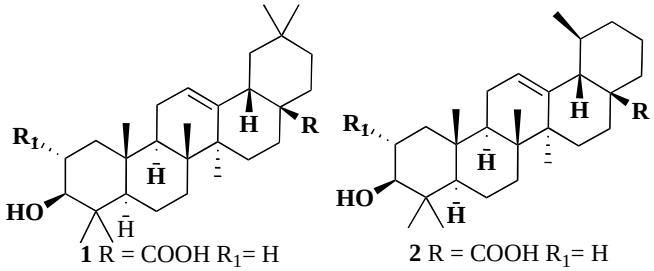
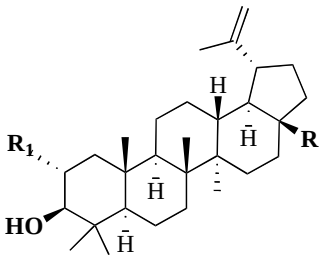


1 R = COOH R_1= H
4 R = CHO R_1= H
7 R = COOH R_1= OH

2 R = COOH R_1= H
5 R = CHO R_1= H
8 R = COOH R_1= OH



3 R = COOH R_1= H
6 R = CHO R_1= H
9 R = COOH R_1= OH
21 R = Me R_1= H

industry and the search of some sectors of chemical industry for bioactive products obtainable from renewable resources. Of course this is not an exhaustive review on the topic, although some literature references to related articles are included.

2. BIOACTIVE NATURAL PRODUCTS IN VEGETABLE WASTE

2.1. Antiproliferative Tterpenoids from Almond Hulls

Almond [*Prunus dulcis* (Mill)] is a well-known tree, grown largely in the USA and Europe. Almond nuts are used as snacks or ingredients for processed foods (bakery, confectionery and others). The world production is estimated at 1,648,916 metric tons (Mt) in 2005. United States is the main producer (681,744 Mt), while European production was estimated at 397,000 Mt approximately, mainly located in Spain, Italy and Greece [5]. Italian production is around 118,000 Mt and is concentrated in the South, particularly in Sicily (which accounts for 70%). Shelled almond production affords hulls and shells as waste products; in addition, skins are obtained from the production of peeled almonds. More than 600.000 Mt/year is the estimated global amount of hulls from agro-industry [6]. At present, almond by-products are not exploited, apart from a limited production of active charcoal from shells, which are mainly employed as fuel material. Hulls and skins are generally discharged or deposited for composting.

In our work on almond hulls [7] we prepared an extract with ethyl acetate (EtOAc) from dried and ground almond hulls. The residue was extracted with ethanol (EtOH) and both crude extracts were submitted to the MTT bioassay [8] for evaluation of antiproliferative activity towards MCF-7 cells (mammary carcinoma). Only the EtOAc extract resulted active and was further fractionated through Si-gel chromatography; pooled fractions A, B, C, D, E and F were submitted to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] bioassay. Fractions A, B and C were substantially inactive; fraction F showed a mild activity, whereas fractions D and E were highly active. Thus, the main constituents of the active fractions were carefully purified and identified through spectral analysis, including MS (Mass Spectrometry), NMR (Nuclear Magnetic Resonance) and optical rotation, aided by literature search. The main components of fractions D, E and F are triterpenoids, mainly the oleanolic (**1**), ursolic (**2**) and betulinic (**3**) acids, and, as minor constituents, the related aldehydes **4** - **6**. We identified also further minor constituents of fractions E and F: these are the related 2-hydroxy analogues of the above cited triterpenoid acids: namely, maslinic (**7**), corosolic (**8**) and alphitolic acid (**9**). In addition, we isolated daucosterol (β-sitosterol glucoside, **10**) from the more polar subfractions of fraction F. All the isolated compounds, and the inseparable mixtures of corosolic/maslinic acids and oleanolic/ursolic aldehydes were subjected to the MTT bioassays against MCF-7 carcinoma cells to determine the GI_{50} values, reported in Table **1**.

Table 1. Growth Inhibition (GI_{50}) of Compounds 1 – 10 **Against MCF-7 Cells***

Compound	GI_{50} (μM±SD)
1	263 ± 25
2	178 ± 15
3	0.27 ± 0.03
4+5	202 ± 19
6	181 ± 20
7+ 8	296 ± 28
9	324 ± 30
10	239 ± 15
5FU[#]	5.34 ± 0.4

*Mammary Carcinoma Cell Line, Michigan Cancer Foundation (MCF).
[#] 5-Fluorouracil

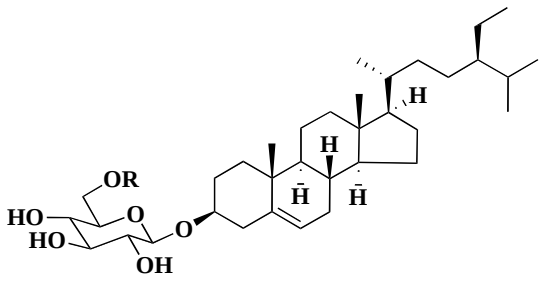
The majority of the compounds listed in Table **1** show a moderate antiproliferative activity, with GI_{50} in the range 180 - 330 μM, whereas betulinic acid (**3**), showing a GI_{50} value of 0.27, resulted more active than 5-fluorouracil (5-FU, GI_{50}= 5.34 μM), an anticancer drug added as a positive control. A comparison with betulinic aldehyde and betulin (GI_{50}= 17.00 μM), the alcohol related to betulinic acid, which was also included as a commercial sample, indicates the importance of the carboxylic group for the antiproliferative activity. Oleanolic (**1**) and ursolic (**2**) acids are also known for their hepatoprotective, anti-inflammatory and hypolipidemic properties and have been reported as anti-tumor promotion agents [9]; betulinic acid was already under study as anticancer, anti-HIV and antimalarial agent before our work on almond hulls [10], but the attention towards the biomedical optimization of this terpenoid and the related oleanolic and ursolic acids as anti-HIV agents has rapidly increased in recent times, leading to the discovery of analogues with potent anti-HIV properties. Daucosterol (**10**) is

known for a variety of beneficial properties [11] and has recently been reported as antimutagenic [12]. All these data suggest that almond hulls are a promising source of useful bioactive compounds. We estimated that more than 700 mgs of betulinic acid per kilogram of almond hulls may be obtained; in addition, approximately a total of 4 grams of oleanolic and ursolic acid may be obtained starting from 1 kilogram of almond hulls.

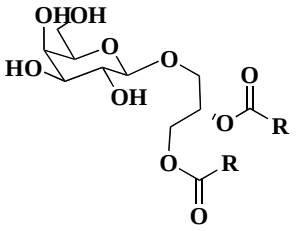
2.2. Antiproliferative Compounds from Grape Stems

Grape is the world's largest fruit crop, with more than 65 million metric tons per year. The European Union affords approximately 30 million tons per year, and the main producers are Italy, France and Spain. In particular, Italy is the first world producer with more than 8.5 million tons per year [5]. Winemaking, that is the production of wine from *Vitis vinifera*, is carried out in different steps: grape collection, destemming, crushing and pressing. From this process mainly two different by-products accumulates: grape stems and grape pomace (skins, seeds and lees). The global amount of grape pomace is approximately 20% of harvested grape. Grape pomace is frequently destined for distillation and production of alcoholic beverages, like *grappa* in Italy. Conversely, there is no real utilization for stems except for composting.

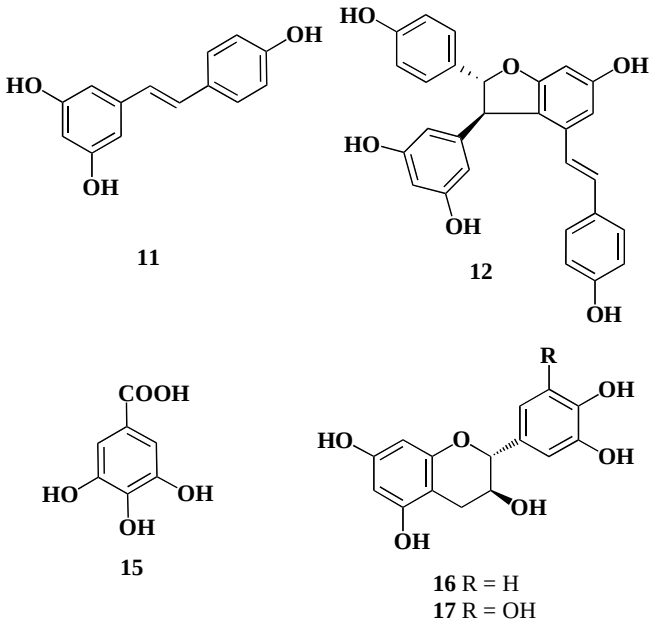
We have examined both grape pomace (see below) and grape stems, this latter material with focus on constituents with antiproliferative activity. In particular, we have examined very recently the EtOAc extract of grape stems obtained from the winemaking of the red grape cultivar 'Nerello Mascalese' (NM), widely cultivated in Sicily on the slopes of the mount Etna [13, 14]. On the basis of the observed activity of the NM crude extract, we carried out a silica-gel gross-fractionation, thus obtaining five pooled fractions A, B, C, D and E. These were submitted to the MTT bioassays on MCF-7 cell line. The active fractions from B to E were carefully chromatographed and the isolated constituents were subjected to spectroscopic analysis (including two-dimensional NMR spectra), as well as to some simple chemical conversions. Among the constituents of the more active fractions B and C we identified, in addition to the above cited oleanolic (**1**) and betulinic (**3**) acids, the stilbenoid *trans*-resveratrol (**11**), and *trans*-ε-viniferin (**12**), a resveratrol dimer. A more polar antiproliferative constituent was found in fraction D: this was shown to be a mixture of sitosterol 6'-*O*-acyl-glucosides (**13a** - **13d**), as proved by GC-MS analysis of the methanolysis products: in fact, the glucose moiety was esterified by the fatty acids linoleic, linolenic, palmitic and stearic (in decreasing percentages). Fraction E, although not very active, was examined for the sake of completeness: also in this case, one of the main products was a mixture of inseparable analogues, namely 2,3-di-*O*-acyl-glicerol galactosides (**14a** – **14e**). Methanolysis followed by GC-MS analysis of the methylesters established the acyl residues, namely linolenic, linoleic, palmitic and stearic (in decreasing percentages). The optical rotation measurement on the galactoglycerol obtained by methanolysis allowed establishing the absolute configuration of the stereogenic centre C-2 as *R* in this product and consequently as *S* in the natural substrate. Further minor constituents of fraction E were identified as daucosterol (**10**), gallic acid (**15**), catechin (**16**) and gallocatechin (**17**). All these compounds, as pure constituents or unseparated mixtures, were subjected to the MTT bioassay on MCF-7 human breast cancer cell. 5-Fluorouracil (FU) was also tested as positive control. The majority of the isolated compounds showed growth inhibitory activity toward MCF-7 tumor cells (GI_{50} < 300 μM). The result confirmed the potent activity of betulinic acid (**3**), resulting more active than the positive control FU against MCF-7 cells. Particularly interesting is the presence of *trans*-resveratrol (**11**), a further constituent with significant antiproliferative activity (GI_{50} = 26 μM). Some other compounds showed a marginal antiproliferative activity, namely ε-viniferin, gallic acid, oleanolic acid and gallocatechin. Compounds **3**, and **11** resulted also highly active towards a CNS glioma cell line (U373-MG) and moderately active against a colon carcinoma cell line (HT-29) and a second CNS glioma cell line (U87-MG). Almost all the constituents identified in Nerello Mascalese grape stems are known in the literature for a number of beneficial properties. Resveratrol (**11**), in particular, is well-known as a grape phytoalexin and considered one of the main phenolic compounds in red wine with chemopreventive properties towards coronary heart disease (CHD) and cancer [15] We have established that resveratrol may be obtained from NM grape stems in approximate amounts of more than 130 mgs per kilogram of dried stems. Betulinic (**3**) and oleanolic (**1**) acids have been already cited above. Sitosterol 6'-*O*-acyl-glucosides **13a**–**d** and 2,3-di-*O*-acyl-glicerol galactosides **14a**–**e** have been previously cited respectively as antiproliferative principles on tumor cells [16] or potent anti-tumor-promoting [17] and anti-inflammatory agents [18]. Gallic acid **15** and the flavonols **16** and **17** are well-known as constituents of black or green tea and reputed to posses antioxidant [19] and anticarcinogenic properties [20].



10 R = H
13 a R = $CO(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$
13 b R = $CO(CH_2)_7(CH{=}CHCH_2)_3CH_3$
13 c R = $CO(CH_2)_{14}CH_3$
13 d R = $CO(CH_2)_{16}CH_3$



14 a R = $CO(CH_2)_7(CH{=}CHCH_2)_3CH_3$
14 b R = $CO(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$
14 c R = $CO(CH_2)_{14}CH_3$
14 d R = $CO(CH_2)_{16}CH_3$



2.3. Antioxidant Polyphenols from Grape Pomace

In a first step of our work on grape by-products, we analysed grape pomace extracts with the aim to isolate potentially useful compounds or obtain extracts or enriched fractions with antioxidant (radical scavenging) properties. The above cited cultivar 'Nerello Mascalese' (NM), was the first to be examined. [13] The MeOH extract obtained from destemmed grape pomace was fractionated and analysed through HPLC-UV-DAD and HPLC-MS-ESI, allowing identification of the main flavonols, flavonols glucosides, flavanols and their gallate esters, anthocyanins, and low-molecular weight proanthocyanins. Five pyranoanthocyanins, less common compounds normally found in aged wines, were also identified for the first time in grape pomace. Quercetin 3-*O*-glucoside (**18**) and quercetin 3-*O*-glucuronide (**19**) resulted the most abundant flavonol glycosides, and malvidin 3-*O*-glucoside (**20**) the main anthocyanin. Flash-chromatography of the EtOAc extract allowed isolation and spectral identification of the triterpenes lupeol (**21**) and oleanoic acid (**1**), the flavonol quercetin (**22**) and daucosterol (**10**). The majority of polyphenols identified are well known for interesting biological properties, especially for their antioxidant and radical scavenging activity [21-23]. In particular, it has been reported that the anthocyanin fraction from an Italian red wine was the most effective phenolic fraction in scavenging reactive oxygen species (ROS) and in inhibiting lipoprotein oxidation and platelet aggregation [24]. In recent studies lupeol is reported as anti-inflammatory [25], inducer of apoptosis [26], and inhibitor of tumor promotion [27]. Oleanoic acid (**1**) and daucosterol (**10**) have been cited above.

As a continuation of this study we have recently examined grape pomace samples from five Sicilian red grape cultivars (Nero d'Avola – NA; Nerello Mascalese – NM; Nerello Cappuccio – NC; Frappato – FR; Cabernet Sauvignon - CS) [28]. The MeOH extracts obtained from grape pomace samples were evaluated for their DPPH• [29] and ABTS•$^{-}$ [30] radical scavenging capacity, and submitted to HPLC-UV-DAD and HPLC-MS-ESI analysis to determine the main

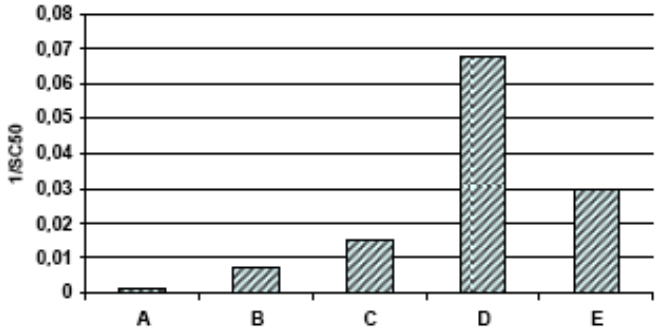


Fig. (1). Antioxidant activity in the DPPH assay of fractions A - E.

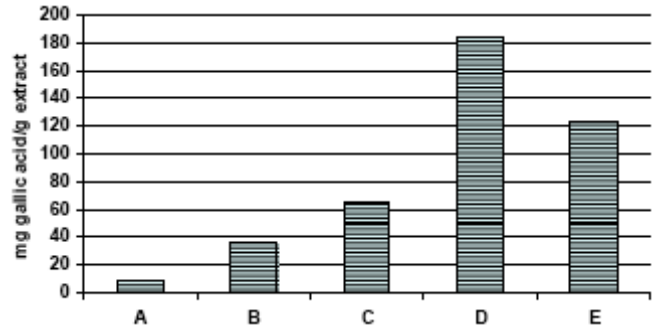
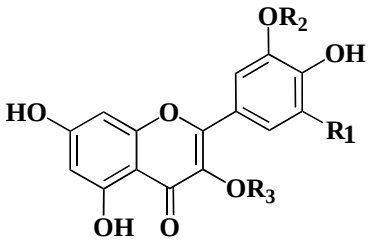


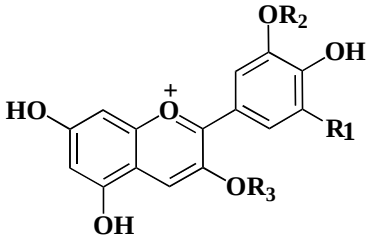
Fig. (2), Total polyphenols (GAE) of fractions A – E.

polyphenolic constituents, namely anthocyanins and flavonols/flavonol glycosides. All the MeOH extracts showed significant antioxidant activity, with some differences between the two methods employed. The NM sample resulted the most active in both tests. A large variability in the total anthocyanins and flavonols content of the MeOH extracts, as well as in the quantitative distribution of the single anthocyanins and flavonols was observed. The most active sample NM showed the highest content in anthocyanins including a free catechol moiety in their structure, a well-known requisite for antioxidant activity [23, 31, 32] More recently, we prepared a defatted ethanolic (EtOH) extract of NM grape pomace which was submitted to the radical scavenging activity test (DPPH•), showing SC_{50} = 9.9 μg/mL, and to the Folin-Ciocalteu assay [33] for the determination of the total amount of polyphenols, expressed as Gallic Acid Equivalent (GAE = 397.7 mg/g extract). The EtOH extract was analysed by HPLC-UV-DAD to identify the main polyphenolic constituents. Subsequently, the extract was fractionated through polyamide chromatography and the pooled fractions A, B, C, D and E were submitted to the DPPH• and GAE tests. The results are reported in Figs. (**1** and **2**).

The antiradical activity is here reported as the reciprocal of SC_{50}, to allow and easy comparison between DPPH• and GAE data; these are well-correlated (R^2 = 0.89) and indicate that the most active fraction D also contains the highest amount of polyphenols. This accounts for only 1.3% weight of the extract; in fact the weight of the inactive fraction A resulted close to 70% of the total eluate: that is, inactive substances are concentrated mainly in this fraction. Notwithstanding the small weight percentage, fraction D has a GAE value (184.1 mg/g fraction D) approximately one half of that of the crude extract; more interestingly, it has SC_{50} = 14.8, that is 67% of the anti-radical activity of the extract. This



18 R_1 = H R_2 = H R_3 = Glc
19 R_1 = H R_2 = H R_3 = GlcA
22 R_1 = H R_2 = H R_3 = H
24 R_1 = OH R_2 = Me R_3 = Glc
Glc = Glucose; GlcA = Glucuronic Acid



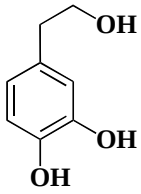
20 R_1 = OMe R_2 = Me R_3 = Glc
23 R_1 = OH R_2 = H R_3 = Glc
25 R_1 = H R_2 = H R_3 = Glc
Glc = Glucose

means that the polyphenols with higher anti-radical power are selectively retained in this fraction. The quantitative analysis of the main anthocyanins and flavonols shows that delphinidin 3-*O*-glucoside (**23**), myricetin 3-*O*-glucoside (**24**), quercetin 3-*O*-glucoside (**18**) and quercetin (**22**), all bearing a catechol moiety, have the highest *relative* percentage in fraction D. With respect to the crude EtOH extract, these flavonoids are also consistently enriched, whereas a significant reduction in peonidine 3-*O*-glucoside (**25**) and malvidin 3-*O*-glucoside (**20**), lacking of the *ortho*-dihydroxy group, is observed.

3. CHEMICAL AND/OR ENZYMATIC MODIFICATION OF BIOACTIVE COMPOUNDS

3.1. Enzymatic Modification of Hydroxytyrosol

According to the second methodology that we have used in exploiting vegetable by-products, we report below a brief account of our recent work on chemical and/or enzymatic modification of some natural 'lead compounds' which may be obtained from vegetable waste. One of these is the simple phenol of olive oil, hydroxytyrosol (**26**), which is formed largely by hydrolysis of the glycoside oleuropein, the bitter principle of the fruit of olive tree (*Olea europea*), during fruit ripening, crushing and pressing to obtain extra-virgin olive oil. This is widely used as dressing in the Mediterranean diet, whose beneficial health effects have been claimed in a variety of studies [34-36]. In particular, olive oil phenolic constituents have been reported as important agents in chemoprevention of colonrectal carcinogenesis [37]. Among them, hydroxytyrosol is reputed to be a good antioxidant and one of the main cancer chemopreventive principles present



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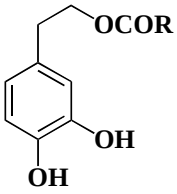


27 R = CH_3
28 R = CH_2CH_3
29 R = $(CH_2)_2CH_3$
30 R = $(CH_2)_8CH_3$
31 R = $(CH_2)_{16}CH_3$

in olive oil, and may be obtained by olive oil mill wastewater [38]. On this basis, we planned to obtain lipophylic analogues of hydroxytyrosol, bearing an acyl chain in C-1 but maintaining a free catechol moiety. We employed a simple methodology based on the use of enzymes in organic solvent. In this case we used a vinyl ester as acylating agent and the enzyme *Candida antarctica* lipase (CAL) in *tert*-butylmethyl ether at 40 °C degrees. We prepared the five analogues here reported and bearing as acyl residue acetate (**27**), propionate (**28**), butanoate (**29**), decanoate (**30**) and stearate (**31**) [39]. These were obtained with very good yields without using heavy metals or noxious reagents, and with a very simple purification step: in fact, the enzyme is simply filtered off the solution at the end of the reaction. The five analogues thus obtained were submitted to the DPPH• radical scavenging activity test and all showed good anti-radical properties, regardless of the length of the acyl chain, as reported in Table **2**. These compounds were also submitted to the 'atypical version' of Comet test [40] to evaluate the protective properties against the oxidative damage caused by hydrogen peroxide treatment on blood cells. Results are reported in Fig. (**3**) histograms reports the TDNA, that is the percentage of fragmented DNA caused by oxidative damage induced by H_2O_2. We determined for hydroxytyrosol and its analogues also the log P value (not reported here), which is related to the lipophylic character of the substance. On the whole, the results showed that all the acylhydroxytyrosols may be profitably used as lipophylic antioxidants, for instance as food or cosmetic additives. In addition, the hydroxytyrosol acetate **27** and propionate **28** with short acyl chains (C_2 and C_3) and log P < 1.2, have good protective properties against DNA oxidative damage; the hydroxytyrosol butanoate **29** (C_4, log P = 1.77) is moderately protective, whereas the decanoate (**30**) and stearate (**31**) derivatives, bearing longer acyl chains (C_{10} and C_{18}) and log P > 5 are almost ineffective. In conclusion, hydroxytyrosol analogues to be studied as drug candidates must have a short acyl chain and a log P lower than 2.

3.2. Chemo-Enzymatic Modification of Resveratrol

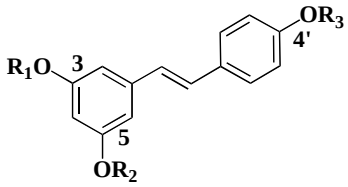
Much of our work on optimization of bioactive compounds identified in by-products has been carried out on *trans*-resveratrol (**11**). This stilbenoid, well-known for a wide range of chemopreventive properties, has recently been evaluated as an antiproliferative agent towards DU-145 prostate tumor cells [41, 42]. Compounds with antiproliferative properties against prostate carcinoma are intensively studied in the hope of obtaining new anticancer drugs or adjuvants of

Table 2. DPPH• Scavenging Activity of Compounds 26 - 31

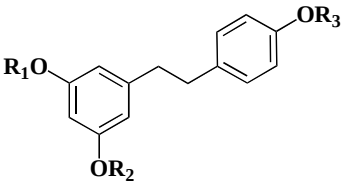
Compound	SC_{50} (μM± SD)*
26	24.6 ± 6.5
27	21.9 ± 1.4
28	22.9 ± 5.1
29	24.7 ± 1.0
30	24.8 ± 10.2
31	20.5 ± 6.6

* SC_{50}, Scavenging Capacity: phenol concentration, expressed in μM, able to quench 50% of DPPH radicals in a 92 μM solution (mAU = 1, solvent: cyclohexane).

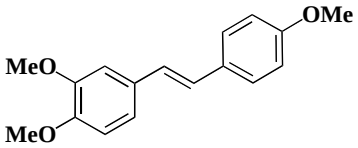
currently used drugs. We planned to obtain lipophilic analogues through a regioselective enzymatic acylation of resveratrol. Direct acylation in the presence of CAL and vinyl acetate in *tert*-amylic alcohol afforded 4'-*O*-acetylresveratrol (**32**), in 90 h with 40% yield [43]. To obtain a library of resveratrol lipophylic analogues we turned to a different methodology, exploiting the observed regioselectivity of the enzyme towards position 4'. This chemo-enzymatic way is based on a preliminary peracylation by conventional chemical reagents, such as anhydrides or acyl chlorides, followed by regioselective deprotection of the peracylated resveratrol. Resveratrol esters, namely peracetate (**33**), perbutanoate (**34**) and peroctanoate (**35**) were subjected to lipase-catalysed alcoholysis, employing *n*-butanol as nucleophilic reagent. Butanolysis was more rapid but less selective than direct acylation. In the presence of CAL we obtained both 4'-deprotected and 5,4'-deprotected derivatives (and acylated butanol as by-product): in fact, the enzyme works preferentially on groups in C-4', and this allowed us to obtain the 3,5-diacetate (**36**), 3,5-dibutanoate (**37**) and 3,5-dioctanoate (**38**); in addition, these products may in turn become a substrate for the CAL, thus affording the corresponding 3-cetate (**39**), 3-butanoate (**40**) and 3-octanoate (**41**). We carried out further chemical and enzymatic modifications on resveratrol [44]; in order to evaluate the possible role of the central double bond, we hydrogenated **11** to obtain the dihydroresveratrol **42**, which was directly acylated in the presence of CAL to afford **43**; the dihydroresveratrol peracetate **44**, submitted to the enzymatic butanolysis afforded the derivative dihydroresveratrol 3,5-diacetate **45**. To enlarge the library of lipophylic resveratrol analogues we prepared also, by simple chemical conversion, the tri-*O*-methylresveratrol **46** and 3,4'-di-*O*-methylresveratrol **47**. The main product **46** was hydrogenated to obtain compound the dihydrotri-*O*-methylresveratrol **48**. To evaluate the possible role of the position of the methoxy groups, we also synthesized a resveratrol analogue with a modified substitution pattern of the stilbene skeleton, namely the 3,4,4'-trimethoxystilbene **49**. This set of lipophylic resveratrol analogues was tested through MTT bioassay on DU-145 prostate carcinoma cell line (see Table **3**). Nine lipophylic analogues resulted more active than resveratrol against the DU-145 cells; in particular, **46** resulted by far the most potent, with a GI_{50} = 2.92 μM. Other methylated derivatives like the dimethyl resveratrol **47** resulted highly active; the activity of the trimethoxystilbene **49** was comparable to that of resveratrol, thus sug-



32 R_1 = H R_2 = H R_3 = $COCH_3$
33 R_1 = $COCH_3$ R_2 = $COCH_3$ R_3 = $COCH_3$
34 R_1 = $CO(CH_2)_2CH_3$ R_2 = $CO(CH_2)_2CH_3$ R_3 = $CO(CH_2)_2CH_3$
35 R_1 = $CO(CH_2)_6CH_3$ R_2 = $CO(CH_2)_6CH_3$ R_2 = $CO(CH_2)_6CH_3$
36 R_1 = $COCH_3$ R_2 = $COCH_3$ R_3 = H
37 R_1 = $CO(CH_2)_2CH_3$ R_2 = $CO(CH_2)_2CH_3$ R_3 = H
38 R_1 = $CO(CH_2)_6CH_3$ R_2 = $CO(CH_2)_6CH_3$ R_3 = H
39 R_1 = $COCH_3$ R_2 = H R_3 = H
40 R_1 = $CO(CH_2)_2CH_3$ R_2 = H R_3 = H
41 R_1 = $CO(CH_2)_6CH_3$ R_2 = H R_3 = H
46 R_1 = CH_3 R_2 = CH_3 R_3 = CH_3
47 R_1 = CH_3 R_2 = H R_3 = CH_3



42 R_1 = H R_2 = H R_3 = H
43 R_1 = H R_2 = H R_3 = $COCH_3$
44 R_1 = $COCH_3$ R_2 = $COCH_3$ R_3 = $COCH_3$
45 R_1 = $COCH_3$ R_2 = $COCH_3$ R_3 = H
48 R_1 = CH_3 R_2 = CH_3 R_3 = CH_3



49

gesting that the substitution pattern is important for the activity. Hydrogenation of the double bond was in some cases scarcely influent on the activity, but dihydrotri-*O*-methylresveratrol **48** resulted significantly less active than **46**. More recently, we found that the trimethylresveratrol **46** (also reported as 3,5,4'-trimethoxystilbene) is a potent inhibitory agent against porcine aortic endothelial cells [45]; the inhibition of angiogenesis in tumor cells is a new frontier in the fight against cancer [46].The promising anti-tumor properties of methylated analogues of resveratrol prompted us to compile a review on this topic [47].

CONCLUSIONS

In conclusion, vegetable waste confirm to be a rich and promising source of bioactive compounds and we tried to exemplify how these constituents may be profitably used employing various chemical protocols. As components of mixtures, such as extracts or enriched fractions, they can be useful, for instance, as food or cosmetic antioxidants; an example is the polyphenols-enriched fraction (1.3% weight but 67% anti-radical activity of the extract) obtained from grape pomace. After purification, bioactive constituents can be employed as components of drugs, beneficial food, etc.;

Table 3. Growth Inhibition of Resveratrol and its Analogues Against DU-145 Cells*

Compound	GI_{50} (μM)
11	24.09 ± 0.4
32	32.18 ± 0.7
33	23.34 ± 0.5
34	23.87 ± 0.5
35	25.07 ± 0.6
36	25.21 ± 0.9
37	19.07 ± 0.9
38	30.82 ± 0.7
39	25.42 ± 0.4
40	21.63 ± 1.1
41	25.20 ± 0.8
42	22.85 ± 0.6
43	23.66 ± 0.6
44	29.76 ± 0.8
45	21.91 ± 1.3
46	2.92 ± 0.9
47	12.24 ± 1.4
48	12.37 ± 0.5
49	25.39 ± 0.5

* Human prostate tumour cell line

worth noting here is the amount of betulinic and oleanolic acids available from almond hulls. Finally, some of these compounds, as the above cited examples hydroxytyrosol (**26**) and *trans*-resveratrol (**11**), can be modified by chemical or enzymatic methods to obtain semi-synthetic analogues with improved biological activity, bioavailability or solubility in a lipophylic medium.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

Financial support for this work was obtained from the University of Catania (PRA, Catania, Italy) and from MIUR (PRIN, Rome, Italy)

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Received: November 12, 2009 Revised: November 16, 2010 Accepted: Febuary 08, 2011