Hydrolyzable tannins from different vegetal species, fractionation HPLC/DAD/MS analyses, and anti-yeast activity

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Abstract. In the present work the quali-quantitative analysis of hydrolysable tannins have been carried out in three different plant species: myrtle, chestnut and pomegranate. A resin fractionation of the aqueous extracts has been also performed to detect the selectivity of the resin towards gallic and ellagic derivatives. The anti-yeast properties of these extracts have been finally investigated.

Introduction. Tannins are distributed in species throughout the plant kingdom, especially in leaf, bud, seed, root and stem tissues, and also in the heartwood of conifers where they may play an important role in inhibiting microbial activity. In the hydrolysable tannins (HTs), usually *D*-glucose is partially or totally esterified with phenolic groups such as gallic acid (gallotannins, GTs) or ellagic acid (ellagitannins, ETs). HTs are hydrolyzed by weak acids or bases to produce carbohydrates and phenolic acids; they are more easily oxidized than condensed tannins (CTs). In the present work, several extracts enriched in HTs, obtained from plants such as chestnut (*Castanea sativa* Mill.), myrtle (*Myrtus communis* L.) and pomegranate (*Punica granatum* L.), have been analysed and characterised by HPLC/DAD /MS methods. The same samples were considered for their antimicrobial activity and compared to the activity of a CTs commercial grape seed extract.

Materials and methods. *Punica granatum* L. fruits were collected in Grosseto, Italy, in September, 2009; *Myrtus communis* L. samples were collected in Isola d'Elba, Italy, in August, 2009; the extract of *Castanea sativa*, M. bark was the commercial Saviotan® by Nuova Rivart Srl. The extracts of pomegranate peel and myrtle leaves were prepared in hot water (15% p/V). Resins used for fractionation are Purolite® MN 202 (Purolite Ltd.) and Amberlite XAD-7 HP (AcrosOrganics). The HPLC/DAD/MS analysis were conducted using a HP-1100 liquid chromatograph equipped with a DAD detector and a HP 1100 MSD API-electrospray (Agilent Technologies) operating in negative ionization mode. A four-step linear solvent gradient starting from 100% H_2O up to 100% CH_3CN was performed with a flow rate of 0.8 mL min^{-1} during a 55 min period. The column was a Luna C18 250×4.60 mm, 5µm (Phenomenex) operating al 26°C. The eluent was H_2O adjusted to pH 3.2 by HCOOH/ CH_3CN. Mass spectrometer operating conditions were: gas temperature 350°C at a flow rate of 10.0 L min^{-1}, nebulizer pressure 30 psi, quadrupole temperature 30°C and capillary voltage 3500 V.

Results and Discussion. The investigated vegetal matrices contain HTs, gallic acid and ellagic acid derivatives. In Figure 1, the chromatogram of a pomegranate peel extract is shown with the list of the detected compounds.

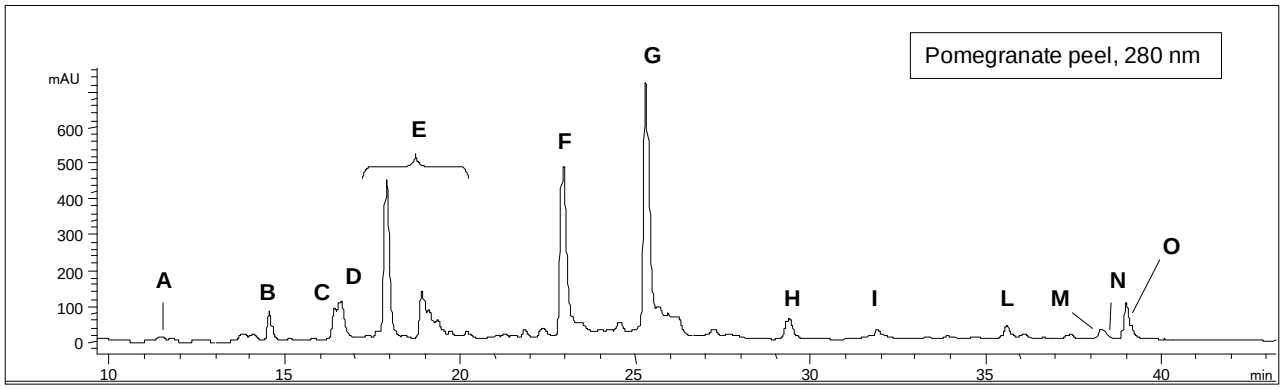

Figure 1. Chromatographic profile of a pomegranate peel aqueous extract. *Peaks:* A. HHDP-glucose; B. Gallic acid; C/D. α/β – punicalin; E. Ellagitannins m/z 1083; F. α-punicalagin; G. β-punicalagin; H. Gallotannin m/z 633; I. Ellagic acid esoside; L. Gallotannin m/z 951; M. Ellagic acid rhamnoside; N. Ellagic ac. pentoside; O. Ellagic ac.

The HPLC/DAD quantitative analysis has been carried out using specific calibration curves, building with gallic and ellagic acid, respectively. The polyphenol concentration ranged from 15 to 75% for the

HTs extracts; the CTs commercial extract had a concentration of 90% in catechins. The possibility of fractionating the tannins contained in the extracts by using crosslinked polystyrenic and acrylic resins has been also evaluated. In particular, MN202 and XAD-7 HP resins have been employed. In the case of myrtle leaves, the main components of the aqueous extracts besides tannins (specially GTs) are flavonols derived from myricetin [1]; the extracts obtained from chestnut contain gallic and ellagic tannins, typically castalagin, vescalagin and pedunculagin [2]; the pomegranate peel extracts also contain gallic and ellagic tannins, especially α and β-punicalagin, α and β punicalin (Figure 1).

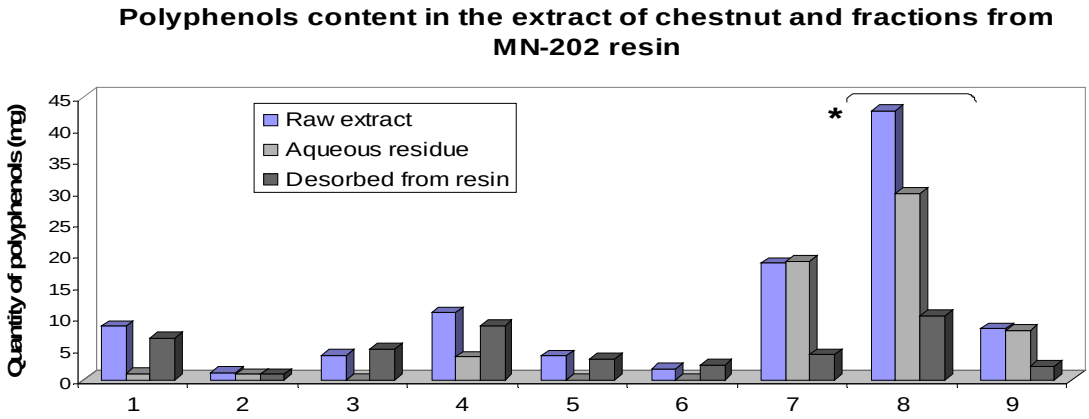

Figure 2. Quantity of polyphenols (data expressed in mg) in chestnut raw extract, resin desorbing ethanol and aqueous residue. *Compounds*: 1. Gallic acid; 2. Monogalloyl-glucose; 3. Digalloyl-glucose; 4. Trigalloyl-glucose; 5. Tetragalloyl-glucose; 6. Pentagalloyl-glucose; 7. Galloyl HHDP-glucose; 8. Castalagin/Vescalagin. ***** For these compounds the graph scale is reduced to 50%; 9. Ellagic tannins.

As shown in Figure 2, the MN-202 resin has a good selectivity towards GTs with respect to ETs of the chestnut aqueous extract. MN-202 resin allows also the removal of the main flavonoid portion from the myrtle leaf extract, with only a partial specificity towards the tannin component (Figure 3). Therefore, the employment of this resin could be useful to obtain fractions enriched with specific polyphenol classes or composed by a single class, as in the case of the aqueous residue obtained from the raw leaf extract of myrtle. In Figure 3 the behaviour of XAD-7 HP resin is also reported and it seems to be less specific toward flavonoids, but it allows to obtain fractions mostly enriched in tannins (Figure 3). Better results could be also obtained using both resins serially.

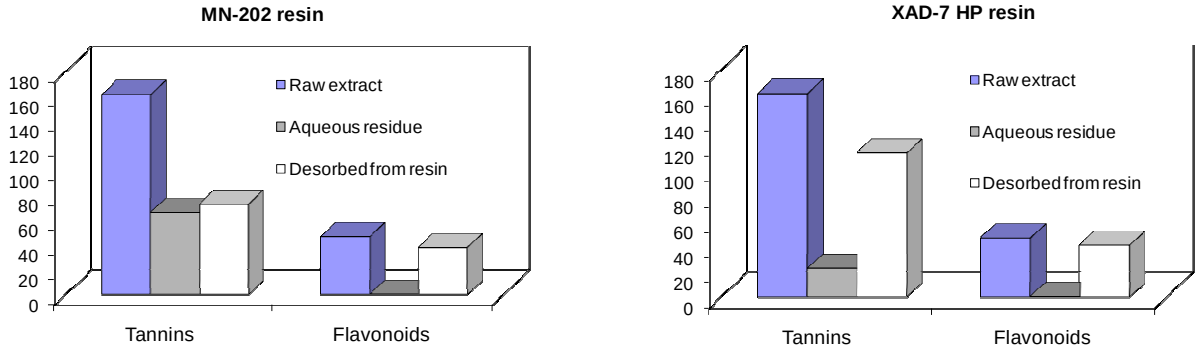

Figure 3. Content of tannins and flavonoids (expressed as mg) in different fractions obtained by using MN-202 and XAD-7 HP from a leaf extract of *Myrtus communis* L..

The scientific and commercial interest in discovering novel active compounds (potentially supporting or even substituting molecules of current clinical use) has oriented the work toward developing novel HTs extracts exhibiting antioxidant and antimicrobial activity [3, 4]. In this work the activity and synergistic effects of amphotericin B, hydrolysable and condensed tannins have been evaluated against yeasts of the species *Candida albicans*, *C. sinensis*, *C. glabrata* and *Issatchenkia orientalis*. The combination amphotericin B (AMB) + hydrolysable tannins exhibited synergy against all *C. albicans*, *C. glabrata* and

I. orientalis strains. MICs on *C. sinensis* of condensed tannin extracts ranged from 200 and 4000 µg extract/mL. These results suggest that the combination of AMB and tannins could be of interest for their potential exploitation in pharmaceutical sciences.

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[3] Buzzini P., Arapitsas P., Goretti M., Branda E., Turchetti B., Pinelli P., Ieri F., Romani A., *Mini Rev Med Chem*, **2008**, *8*, 1179-1187.
[4] Romani A., Ieri F., Turchetti B., Mulinacci N., Vincieri F.F., Buzzini P., *J. Pharmac. Biomed. An*., **2006**, *41*, 415-420.