Molecular investigation of valonea tannin

by

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ABSTRACT

Valonea is one of the well-known vegetable tannins, have been used for tannage and retannage processes in leather making. Besides being used very extensively in leather industry, its main components and chemical structure is gradually identified. In order to investigate the chemical structure of valonea with novel techniques, MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight) and FTIR (Fourier Transform Infrared) Spectroscopy were used. MALDI-TOF spectrum showed the presence of low molecular weight fraction of hydrolysable tannins like nonahydroxytriphnoic, flavogallonic acid, ellagic and gallic acid, pentagalloylglucose and all sorts of degradation and oxidation products are thought to be major components of valonea tannin. Moreover, castalagin/vescalagin and ones added by a few residual structures/atoms in this way having larger molecule and bigger mass than castalagin/vescalagin, derived by internal rearragements of a larger molecule are major components of valonea. From the FTIR spectrum of valonea, it is detected that valonea has a complex structure having -OH at 3420 cm^{-1}, C=C at 1610 cm^{-1}, C=O at 1734 cm^{-1}, CH_2 at 2939 cm^{-1}, C-OH at 1340 cm^{-1}, C-O-C at 1185 cm^{-1} and ester bonds at 1045 cm^{-1}.

INTRODUCTION

Leather industry is looking at options for metal-free tanning systems and especially tanning materials based on the natural products such as vegetable tannins are gained importance. It is known that plants synthesis different polyphenolic substances, some of which may contribute to the formation of tannins. Tannins are one of the many types of secondary compounds found in plants and widely distributed in the plant kingdom[1].

Vegetable tannins are one of the oldest materials used for tanning hides and skins. White[2] defined the term tannin as the substance which converts the putrefiable hide or skin into imputrescible leather. Probably the most acceptable and simple definition for tannins is that of Bate-Smith and Swain “water soluble phenolic compounds having molecular weights between 500-3000 and, besides giving the usual phenolic reactions, they have special properties such as the ability to precipitate alkaloids, gelatin and other proteins”[3].

According to their chemical nature and structural characteristics vegetable tannins are subdivided in two groups[4]:

- Condensed tannins
- Hydrolysable tannins

Chestnut, tara, valonea, sumach, divi divi, algarobilla and myrabolans are the most common used hydrolysable tannins in leather industry.

Valonea tannin, obtained from tannin rich fruits of acorn cups of *Quercus* species, have been used to tan hide and skin either alone or accompanying with other tanning agents in Turkish leather industry extensively. Besides giving excellent firmness and fullness and better light fastness and lower water absorption properties to leather then many other vegetable tannins, the use of valonea in the tanning process represents some problems such as sludge formation in the process pits, high astringency of tanning, and unfavorable color of leather[5].

The TEMA Foundation (non-profitable organization dedicated itself reforestation and protection of natural habitats in Turkey) has started campaign for plantation of 10 billion oak trees including tannin rich *Quercus* species all over Turkey. When the project is ended, huge amount of raw materials will be available for valonea extraction. For this reason, a research project has been started to improve the properties of valonea in order to extent its use to worldwide tanning industry.

The first part of this project is aimed to investigate valonea tannin in the molecular level by using the rather novel technique called matrix-assisted laser desorption/ionisation-time-of-flight mass spectrometry (**MALDI-TOF-MS**) and **FT/IR** spectroscopy.

MATERIALS AND METHODS

Material

Acorn cups and beards used for extraction were obtained from fruits of oak trees growing up around Salihli-Manisa-TURKIYE.

Method

EXTRACTION:

Dried crude sample (100 g cups and beards) was cut in to small pieces. Then the crude sample was taken in to koch extractor and extracted with deionised water at 70 ^{o}C in 4 hours. The extract was concentrated under pressure at 50 ^{o}C. Concentrated extract was dried by using LAB-PLANT SD-04 spray drier.

MALDI-TOF-MS:

The sample was dissolved in acetone (4 mg/ml). Then the sample solution was mixed with an acetone solution (10 mg/ml) of the matrix, for which 2,5 dihydroxy benzoic acid was used. The solutions of the sample and the matrix were mixed in equal amounts and 0.5 to 1 µl of the resulting solution was placed on the MALDI target. After evaporation of the solvent, the MALDI target was introduced in to the spectrometer. The spectra were recorded on a Kratos Kompact MALDI 4 instrument (Kratos Analytical Instruments, Ramsey, NJ). The irradiation source was a pulsed nitrogen laser with a wavelength of 337nm. The length of one laser pulse was 3 ns. The measurements were carried out using the fallowing conditions: positive polarity; linear flight path; high mass (20 kV acceleration voltage); 100-150 pulses per spectrum. The delayed extraction technique was used, applying delay times of 200-800 ns[6].

FTIR:

FTIR spectra were recorded on a Perkin Elmer-Spectrum Bx FT-IR System spectrophotometer, in a matrix of KBr (0.5-1 mg tannin sample in 100-200 mg dried KBr).

RESULTS AND DISCUSSION

From the spectrum obtained for the valonea extract, mass increments of roughly 170-Da, 302-Da and 471-Da were found in the spectra and structures given in Table-I were detected (see Fig.1-4). In addition mass peaks for ellagic acid at 326-Da, flavogallonic acid at 492-Da and nonahydroxytriphnoic acid at 523-Da were found in the spectra. These chemical species are the characteristic of the low molecular weight fraction of hydrolysable tannins as cited before by Tang at all[7].

Table I

Mass peaks detected in valonea

Base Peak	**B**	**C**	**C**	**C**
(A)	A + 302 Da	B + 170 Da	B + 302 Da	B + 471 Da
297	601	769	---	1071
311	616	---	---	1088
340	643	816	---	1109
355	655	825	959	1127
371	673	844	976	1145
394	693	---	995	1168
409	710	---	1012	1181
429	731	---	1033	1209
448	750	---	1051	1219

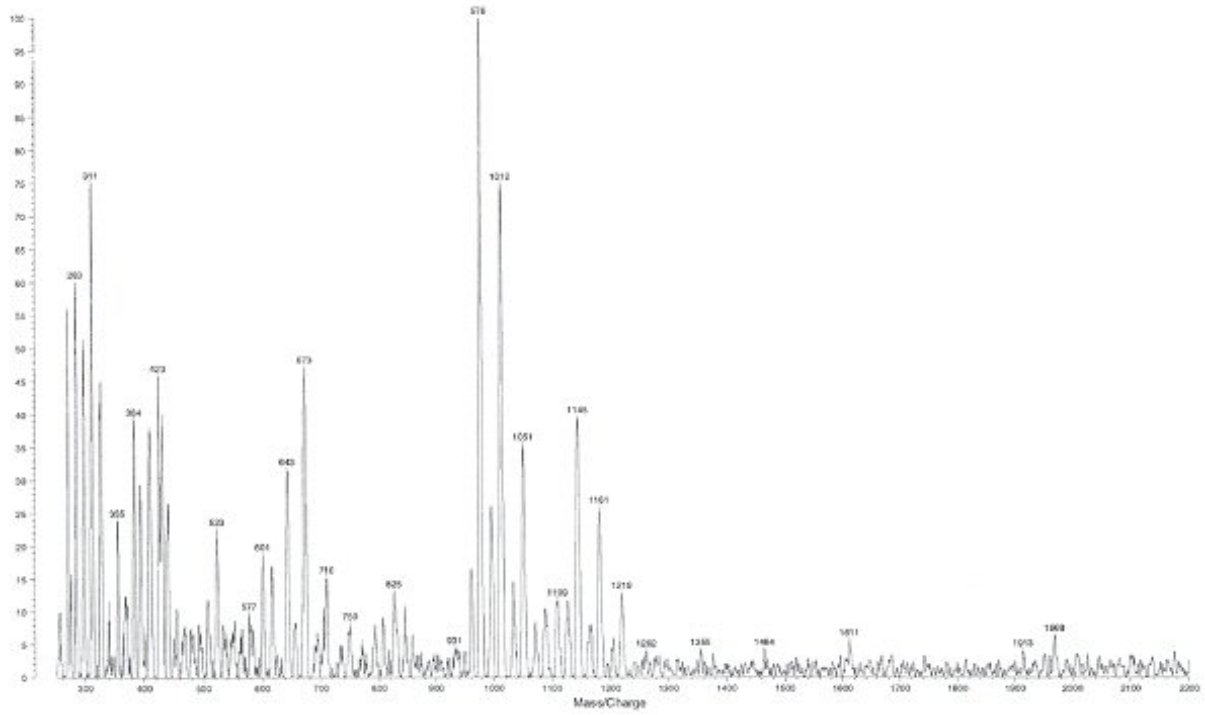

Fig.1. MALDI mass spectrum of valonea

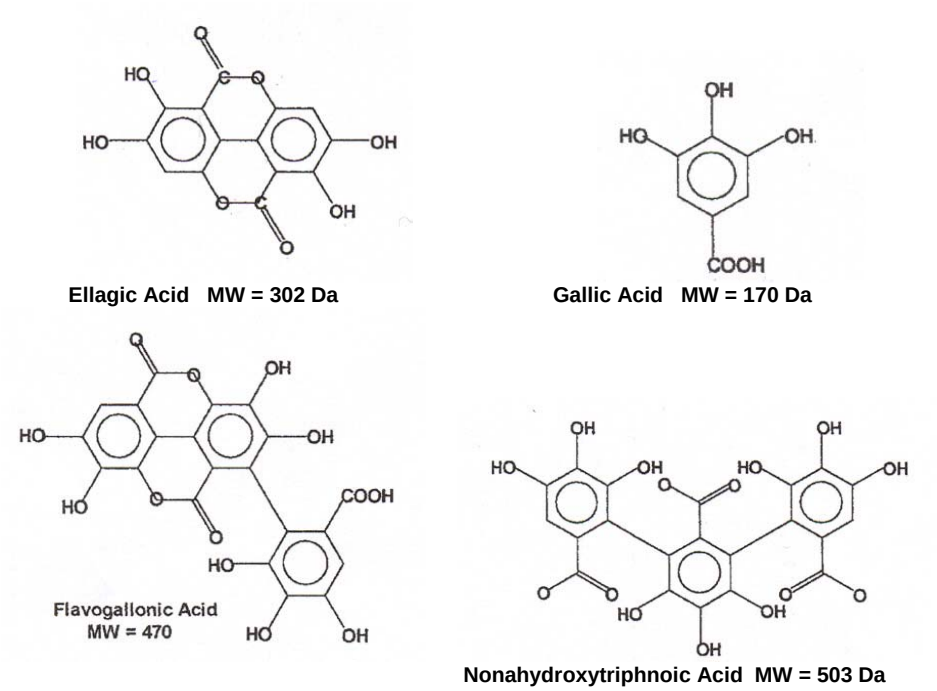

Fig.2. Low molecular weight fragments of valonea tannin extract

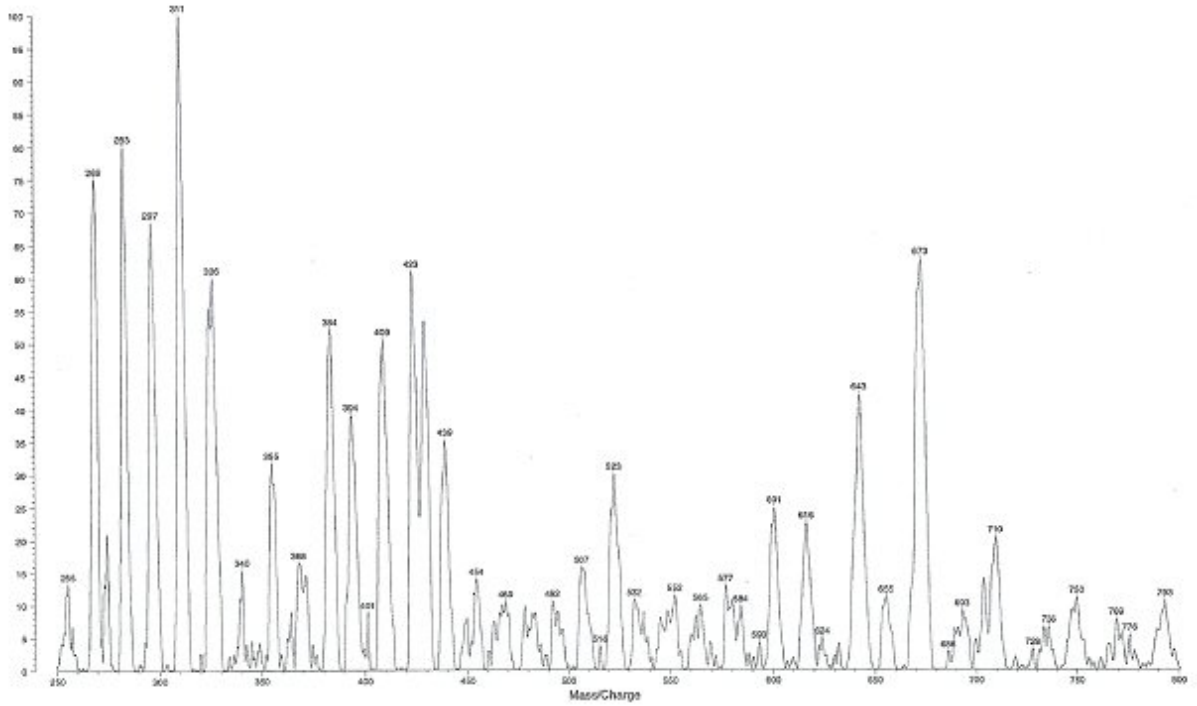

Fig.3. Details of the 250-800 Da range of valonea mass spectrum

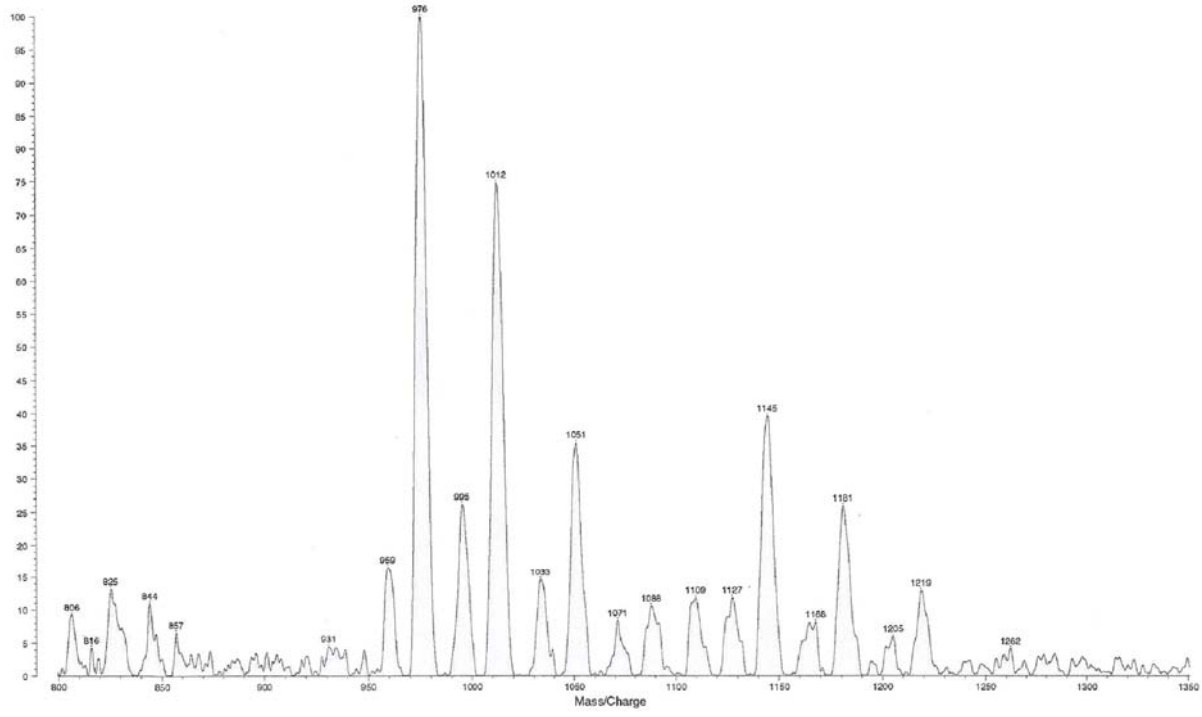

Fig.4. Details of the 800-1350 Da range of valonea mass spectrum

655, 673 and 769 peaks are respectively obtained from the 959-Da, 976-Da and 1071-DA peaks by loss of an ellagic acid structure. The peaks at 959 and 976 are, respectively, the 935-Da castalagin or pentagalloylglucose to which has been added a C-O-C grouping from a group that has split of (976) (Figure 5) and a 935-Da to which, again, a C-O-C remains attached and an –OH has been subtracted (959).

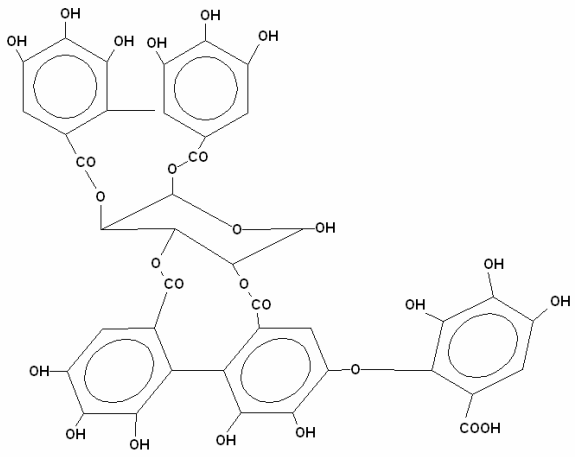

Figure 5. One of the main constituent structure of valonea (959 and 976-Da)

Another major peak at 1012 is thought to be corresponding the structure that a glucose core holding a favogallonic acid (470-Da), an ellagic acid (302-Da) and a gallic acid. The 1088 peak corresponds to either a castalagin (935-Da) structure added by a gallic acid residue that esterifies the only free alcoholic –OH group of the structure. The 1071 peak is the same with 1088 peak by the loss of an –OH group. The 1109-Da peak is the same with 1088 peak but more likely presents an –OH group. The 1127 peak would be vescavaloneicacid/castavaloneicacid (Figure 6). The peak 1219 corresponds to either a castalagin/vescalagin (935-Da) structure added by a ellagic acid (302-Da) with loss of an –OH group from the structre.

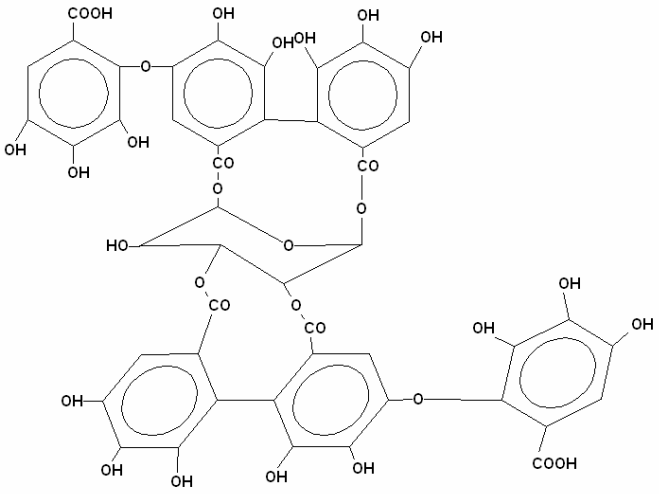

Figure 6. Vescavaloneicacid/Castavaloneicacid (1127-Da)

Evaluation of the findings showed us that valonea has similar structural configuration with chestnut tannin considering Pash and Pizzi's study results except that chestnut has tridimensional macromolecular chains[6]. This means that pentagalloylglucose, castalagin/vescalagin and their rearranged derivatives although reputed to be one of the main constituents of valonea extract.

FTIR spectroscopy

As it is known FTIR is used to determine characteristics of a compound which depend on its functional groups appear in the FTIR fingerprint region. As pointed out by Nakagawa and Sugita it is believed that characterization of vegetable tannins by spectroscopy stands a good chance of success and in the study results they have carried out, they cited that FTIR spectra of each tannin showed characteristic absorption patterns, which makes it possible for us to characterize each tannin[8].

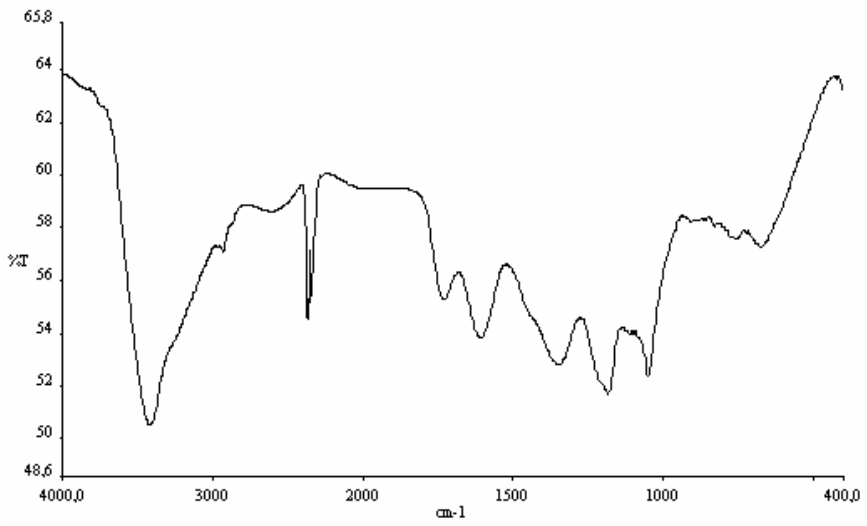

Fig.4. FTIR spectrum of valonea tannin extract

From evaluation of FTIR spectra of valonea presence of -OH at 3420 cm^{-1}, C=C at 1610 cm^{-1}, C=O at 1734 cm^{-1}, CH_2 at 2939 cm^{-1}, C-OH at 1340 cm^{-1}, C-O-C at 1185 cm^{-1} and ester bonds at 1045 cm^{-1} are detected (Fig.4). From the results it is clearly seen that valonea tannin has very complex structure.

CONCLUSION

Evaluation of the results obtained from MALDI spectra of valonea, it is clearly seen that valonea has the same chemical species that characteristic of the low molecular weight fraction of other hydrolysable tannins as cited before by Tang et al.. Most of these low molecular weighted structures thought to be extraction-induced degradation products of valonea tannin. Higher molecular weight structures detected in valonea especially pentagalloylglucose and all sorts of degradation and oxidation products of it are thought to be major components of

valonea tannin. Some of the other molecular structures determined in tannin are thought to be related with internal rearrangements of degradation products with higher molecular structures. But most of these structures are indeed castalagin/vescalagin ones added by a few residual structures/atoms in this way having larger molecule and bigger mass than castalagin/vescalagin, derived by internal rearragements of a larger molecule. Additionally by the FTIR spectrum of the valonea it is detected that valonea has a complex structure having -OH at 3420 cm^{-1}, C=C at 1610 cm^{-1}, C=O at 1734 cm^{-1}, CH_2 at 2939 cm^{-1}, C-OH at 1340 cm^{-1}, C-O-C at 1185 cm^{-1} and ester bonds at 1045 cm^{-1} holding the structure.

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