

# Identification of Gentisic Acid in Cupules of Oak (*Quercus branti* var. *persica*)

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**ABSTRACT:** Oak tree (*Quercus persica*) is a native plant genus in some areas of Iran, spatially in the western and the northwestern regions. This tree is a rich source of phenolic compounds such as gallic acid and tannin. Phenolic compounds present in spice plants as dietary sources possess bioactive properties protecting cellular systems against oxidative stress. For identification of Gentisic acid (2,5-dihydroxybenzoic acid) in cupule of oak, cupules were harvested from a random sample of plants in 3 replications in Lorestan province then put in bags and dried in shadow. Extract was thickener by using Rotary apparatus. Finally, determination and identification of Gentisic acid was by High-performance liquid chromatography (HPLC) method.

**Keywords:** cupule of oak, Gentisic acid, HPLC, *Quercus persica*.

## INTRODUCTION

Phenolic compounds are a large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants. These phenolics are the most abundant secondary metabolites in plants and can be classified into nonsoluble compounds such as condensed tannins, lignins and cell-wall bound hydroxycinnamic acids, and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids and quinones (Harborne and Williams, 2000). Phenolic compounds exist in foods and beverages in various chemical forms, and chemical form determines their gut absorption. Chemical structure will also influence the conjugation reactions with methyl, sulphate or glucuronide groups and the nature and amounts of metabolites formed by the gut microflora and absorbed at the colon level. Understanding the structural factors influencing absorption and metabolism is essential to determine phenolic compounds that are better absorbed and that lead to the formation of known active metabolites (Stankevicius et al., 2010).

Phenolic compounds are abundant in wines and play an important role in controlling oxidation in the human body. They possess reported to have anticancer and anti-inflammatory effects *in vitro*, as well as the ability to block cellular events predisposing to atherosclerosis and coronary heart disease (Tian et al., 2009; Guendez et al., 2005; Monagas et al., 2005 and Teissedre et al., 2000). Gentisic acid (C<sub>7</sub>H<sub>6</sub>O<sub>4</sub>) is a dihydroxybenzoic acid which is a derivative of benzoic acid and a minor (1%) product of the metabolic break down of aspirin, excreted by the kidneys. Gentisic acid is produced by carboxylation of hydroquinone (Tian et al., 2009; Levy and Tsuchiya, 1972). The jungles in west and northwestern Iran that are the main place where oak trees are grown, are 5.2 million hectare big and contain over 49 % of the jungles of Iran. Considering the high expansion of oak trees and the great number of applications of Phenolic compounds specially, Gentisic acid, this research paper is about to find whether there is such a combination in cupule of oak or not.

## MATERIALS AND METHODS

Collecting the samples was done in a village named Dinarvand, with geographical length and width of about 252646, 3696167 meters respectively, and also the altitude of 1452 meters. Three samples were chosen from this area. Each sample containing 10 trees that were healthy from the aspect of pests. To make sure about the

sampling, 3 samples were taken from each tree and then were mixed. While collecting the samples, geographical length, width, and altitude of the GPS, Garmin Vista model, were recorded (Bajalan et al., 2013).

To recognize the best time for collecting the samples we went to the place several times. It was November, when half of the fruits of oak trees were separated from cupules. After sampling, the process of drying was done in the shade, and the room temperature. After drying, the samples were kept in some bags, separately until the time of extracting. In every bag, the name of the area, the place of collecting, and its special number were recorded (Bajalan et al., 2013). In order to extract, first the sample were crushed hefty in order to decrease the volume rather than the level. Then 10 gr of each sample was put in a 1-litre jar and about 100 ml 96% alcohol was added to it. Afterwards the samples were in the room temperature and away from sunlight, for 72 hours. During the time, samples were shaker and mixed for several times. Then in order to thicker the extract, Rotary set was used, and this action (thickening) was done in half an hour. Finally the mere extract was poured in Petri dish, and was tightly covered by its cap, and the features were written on the dish and it was put in the refrigerator till the time of analyzing by HPLC.

For identifying Gentisic acid, HPLC, with standards of 2-5dhy-b-a, choloro-aci, vanilic acid, caffeic acid,3-4dhy-b-a, p-coma-a, syrinigic a and ferulic a in 245- 275- 305- 320 λmax, with the mentioned characteristics in table 1 were used in medicinal plants department of Shahid Beheshti University, Iran.

Table 1. Characteristic of the machine (HPLC)

The characteristics of HPLC	Hplc knauer Autosampler3900 Pumpe1000 PDA Detector 2800
The characteristics of chromatography column	Packing: Eurospher 100-5 C18 Dimension: 250*4.6mm with precolumn
λmax	254-275-305-320
Portable phase	1. methanol + trifluoroacetic acid % 0.02 2. water + trifluoroacetic acid % 0.02
Gradient program	Methanol 20%-90% + trifluoroacetic acid % 0.02 with 70 minutes.
Flow speed	1 ml/min
temperature	25 0C

## RESULTS AND DISCUSSION

Identification the phytochemical compounds of trees and plants that grow locally and knowing about these combinations and applying modern methods to identify and use them are a superiority that should be noticed in assessment of the jungles and pastures. The results of the research show that there is Gentisic acid in cupules of oak (Figure 1).

As a hydroquinone, gentisic acid is readily oxidised and is used as an antioxidant excipient in some pharmaceutical preparations. In the laboratory, it is used as a sample matrix in matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, and has been shown to conveniently detect peptides incorporating the boronic acid moiety by MALDI (Crumpton et al., 2011 and Strupat et al., 1991).

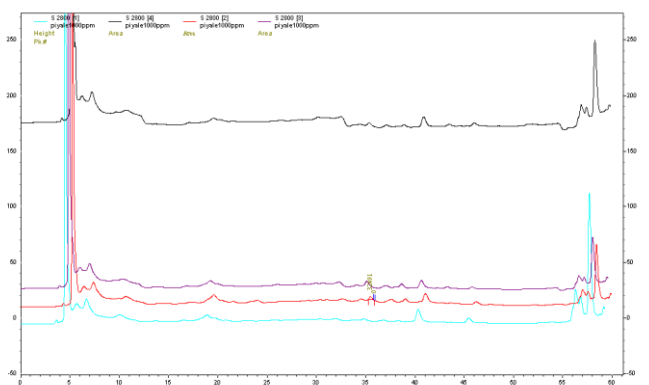


Figure 1. Chromatograms of gentisic acid in Cupules of Oak

By use of the results of this research and other investigations about this tree in different areas and even in deferent kind of *Quercus*, we can recognize the best area from the aspect of this chemical composition. By researching about the morphological and ecological features of the areas were these trees are grown, we can find the relation between these factors and the amount of effective compositions in plants. The characteristics of agrology are another important factor that should be considered. Also different ways of extract and analysis the extracts, are factors that should be noticed. Further information in this area, needs further research.

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