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Investigation of epicatechin in barberry fruits

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Abstract- **Barberry (*berberis vulgaris* L.) belongs to berberidaceae family which is a well known medicinal plant in Iran and has also been used as food. With regards to its medicinal properties it is estimated that this plant had antioxidant properties. Barberry fruits are obtained from khorasgan Bazar and it is green tea leaves from Lahijan fields. The fruit samples were extracted and analyzed by HPLC, using epicatechin standard. The results showed that epicatechin concentration were 473 *mg/kg* whereas this concentration in green tea leaves was 750 *mg/kg*. Because epicatechin is the main catechin compound of green tea and barberry also has epicatechin compound and this has been reported. It is suggested that barberry fruits is a medicinal plant**.

Keywords--component; Medicinal plant, Epicatechin, Barberry

I. INTRODUCTION

Barberry (*Berberis* L.) species are commonly used in nutrition, medicine and homeopathy. They have marked antibacterial and anti-fungal properties as well as hypotensive properties that are attributable to berberine and other isoquinoline alkaloids. *Berberis vulgaris* L. fruits are used for ailments and discomforts of kidneys, urinary and gastrointestinal tract, for liver diseases, bronchial discomforts, and as a stimulant for the circulatory system. The root and stem of these species are used for ailments and complaints of gastrointestinal tract, liver, gallbladder, kidney and urinary tract, respiratory tract, heart and circulatory system, as well as febrifuge. Bark of the root and stem of *B. vulgaris* have both medicinal and toxic properties due to berberine, an isoquinoline alkaloid, mostly present in these organs [6].

Berberis koreana (Korean barberry) is a dense, thorny plant useful for barrier planting. It has red fall foliage and produces yellow flowers in 3- to 4-inch racemes. Traditional medicines have become increasingly popular worldwide, and there is mounting evidence suggesting that medicinal plants could be unlimited reservoirs of drugs. Researchers have intensified their efforts towards the scientific evaluation of traditional medicines. In traditional eastern medicine, extracts of various plants from *berberidaceae* family (*B. aquifolium*, *B. vulgaris* and *B. aristata*) are used for rheumatic complaints and other types of chronic inflammation (1–3). It has been reported that the functional components of the extracts are alkaloids, such as berberine, oxyacanthine, berbamine, and palmatine [10].

On the other hand, leaves, fruit pulp or flowers can contain only traces of that alkaloid. Extracts from root of *Berberis vulgaris* and *B. croatica Horvat* expresses antimicrobial activity against *Bacillus subtilis* NCTC 8236, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 10535, Pseudomonas aeruginosa ATCC 27853 and *Candida albicans* ATCC 10231. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, reactive oxygen species (ROS) that are formed during metabolism, may contribute to pathogenesis of many diseases such as diabetes, cance and cardiovascular diseases. Conversely, some natural compounds, may act as antioxidants that are effective in protecting human body against damage caused by ROS. Among natural antioxidants, phenolic compounds are an object of special interest because of their wide distribution in the plant kingdom. All the phenolic classes (simple phenolics, flavonoids, phenolic acids and anthocyanins) have the structural requirements of free radical scavengers and have potential as food antioxidants. Phenols, especially flavonoids, can also have additional properties like anti-inflammatory, anti-bacterial and anti-fungal effects [6].

Berberis vulgaris L. grows in Asia and Europe as Galbanum [2] and Neem [7]. The plant is well known in Iran and has been used extensively as a medicinal plant and as a food. This genus is a shrub 1-3 m tall, spiny, with yellow wood and obviate leaves, bearing pendulous yellow flowers succeeded by oblong red berries [12]. Barberry Besides medicinal properties [3] has significant levels of antioxidant compounds such as catechin. One of the most important antioxidants is catechin compounds in the green tea in which they significantly high [9, 8] and epicatechin existed in peanut skin [5], strawberry and in all of the vegetables and fruits [1, 14].Because of these information barberry fruits were investigated [13].Barberry is both a medicinal fruit and a food. In order to obtain accurate data the study design will be focused on sampling plan and percision of analytical determination and they were compared with green tea leaves that are full of catechin [4].

A. Need for a biotechnological approach

Biotechnology offers an opportunity to exploit the cell, tissue, organ or entire organism by growing them in vitro and to genetically manipulate them to get desired compounds. Many facets of biotechnological approaches can be envisaged. Since the world population is increasing rapidly, there is extreme pressure on the available cultivable land to produce food and fulfill the needs. Therefore, for other uses such as production of pharmaceuticals and chemicals from plants, the available land should be used effectively. Hence, it is appropriate to develop modern technologies leading to plant improvement for better utilization of the land to meet

the requirements. The development of micro propagation methods for a number of medicinal plant species has been already reported and needs to be adopted. Cryopreservation of cells is an area of importance in the conservation of medicinal plants. It has already been used in many plant species. The development and adoption of plant cell culture and organ culture methods have lead to the production of plant products on a large scale. This has been possible by the combined efforts of cell biologists and chemical engineers. Chemical engineers are currently developing improved and appropriate bioreactors for the improvement of production systems by adopting techniques of growth and metabolite production coupled with downstream processing of the products. The improvements in molecular biological research have given a new dimension to in vitro culture as well as for plant improvement, enhancing the yields of the product and resulting in multiple products or producing novel products from genetically engineered plants. Moreover, the need for safer drugs without side effects has led to the use of natural ingredients with proven safety. These factors have laid emphasis on the use of biotechnological methods to enhance the production of pharmaceuticals and food additives, both in quality and quantity [11].

B. Plant cell culture as a source of secondary metabolites

Plant cell cultures are an attractive alternative source to whole plant for the production of high-value secondary metabolites. Plant cells are biosynthetically totipotent, which means that each cell in culture retains complete genetic information and hence is able to produce the range of chemicals found in the parent plant. The advantages of this technology over the conventional agricultural production are as follows. It is independent of geographical and seasonal variations and various environmental factors. It offers a defined production system, which ensures the continuous supply of products, uniform quality and yield [11].

C. Immobilization of plant cells for the production of secondary metabolites

Improvement in the secondary metabolite production of cell cultures is often associated with the organization and differentiation of plant cells. The concept of organization and differentiation led to the use of immobilization technology, which has long been used for microbes and enzymes. Immobilization is defined as a technique, which confines a catalytically active enzyme or cells on a fixed support and prevents its entry into liquid phase. Immobilized plant cells have been used for single and multistep biotransformations of precursors to desired products as well as for the de novo biosynthesis of secondary metabolites. Immobilization of plant cells has distinct advantages as biocatalyst over the immobilized enzyme system. It is necessary to provide the immobilized enzyme with proper pH, the flow of reaction mixture temperature and as supply of cofactors. Immobilized enzymes are generally applied to single-step reactions. Furthermore, there will be loss in activity during isolation of enzymes from the organism. The advantage of immobilized enzyme is the high rate of activity. In contrast to immobilized enzymes, immobilized cells have distinct advantages: (a) it can carry out multienzyme operations; (b) by selecting highly biosynthetic cells, catalytic activity can be enhanced; (c) there is no need to provide cofactors since cells themselves produce them and (d) immobilized cells can be easily handled as compared to immobilized enzymes. Thus, immobilized cells are gaining much importance as biocatalysts. The following prerequisites are essential to adopt immobilization for secondary metabolite production [11].

II. MATERIAL AND METHODS

The fruits of *Berberies vulgaris* L. are collected from khorasgan and green tea leaves from Lahijan fields. The standard chemicals of epicatechin were purchased from sigma. Methanol, actetonitrile and chloroform were analytical grade and from Duksan company. Twice distilled water was filtered by decompressing pump and filter. The instruments used in this research were as follows a 426 HPLC pump, a 486 detector, a Readyne injection valve. The analytical HPLC column was a water empty column (3/9×300mm), packed with 18 packing material an isocratic mode which was used in mobile phase. 1% acetic acid in water/acetonitrile/ethylactate = 87/10/3 vol% at a flow rate of 1 ml/min. The injection volume was 20µ1 and UV .Wavelength was set at 280 nm .chromate connected to PC was used as a data acquisition system. The extraction was concentrated with a rotary evaporator. Extracted of compounds dried fruits with pure water at 80^{o}C for 4h , respectively under continuous string at 300rpm.The filtered samples were initially partitioned with water/ chloroform (1:1 vol%) .Then the water phase was collected and the impurity associated with the chloroform phase was discarded. As a second partition water /ethylaceatate (1:1 vol%) was used. Catechin compound moved in to the ethylacetate layer and were collected for analysed.

III. CONCLUSION:

Standard curve for Epicatechin with 7.8, 15.8 and 3/05 *mg/kg* concentration draw. Then epicatechin curve for green tea leaves and barberry fruit were compared. Epicatechin concentration for barberry was 473 *mg/k*g and for green tea leaves 750 *mg/kg*. Barberry fruits have a high content epicatechin, this fruit besides used for food as a medicinal plant suggested.

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