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Opuntia ficus indica (Prickly pear): extraction and characterization of products with anti-age and antioxidant activity

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The use and application of natural products in Food and Pharmaceutical industry is of great interest, since the beginning of human history and are the basis of modern medicine. In the present study the ethanolic extraction of *Opuntia ficus indica* is investigated. This plant has been used in traditional medicine because of its role in treating a number of diseases and conditions, including anti-inflammatory effects [1]. Specifically, in this study, the plants were collected and split in two parts. One was consisted of the seeds and the other of the leaves and fruits. Subsequently, was held dry of the different parts of the plant. The method of the extraction process involved maceration followed by percolation and in the end of the process a strict protocol of quality control was applied as also an HPLC analysis. The extracts obtained via the specific extraction can be used either as individual medicinal formulations or in combination with other natural extracts to reestablish the healthy natural functions of the body. The current study, aims to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs from *Opuntia ficus indica*. The most important feature of these productions that all materials and products are non-toxic, natural and friendly to the environment.

Key words: prickly pear, extraction, antioxidant properties,

INTRODUCTION

Opuntia, commonly called prickly pear [2] or nopal fruit, is a genus in cactus family, Cactaceae [3]. Prickly pear is native to North America but it has spread to Central South America, North Africa, Europe, Mediterranean countries, the Middle East, and other countries [4].

The fruit has a unique composition of nutrients, including high levels of vitamin C, B-family vitamins (B2, B3, B6, B9), minerals (Ca, Fe, Mg, P, K, Zn), and soluble fibers, and it can be consumed raw or dried. According to The American Journal of Clinical Nutrition (2004), prickly pears have high levels of flavonoids, polyphenols, and betalains[5]. All the parts (pips, skin, pulp, leaves) of prickly pear are exploitable and have anti-oxidant, anti-inflammatory and anti-aging properties.

The health benefits of prickly pear include its ability to lower cholesterol levels, improve the digestive process, decrease the risk of diabetes, and boost the immune system. The antioxidant-rich fruit also helps strengthen blood vessels, aid in weight loss, reduce inflammation and also protect the skin and lower the chances of premature aging [5].

In this work the medicinal properties of prickly pear are presented; methods of valuable compounds extraction are shown and the products of extraction are compared; extracts characterization and product quality control is demonstrated. Also, the potential uses of valorization of prickly pear fruit waste are referred.

Prickly pear has largely been ignored in the United States as a potential health-improving plant. Although demand for *Opuntia* as a vegetable for its fruit products is increasing (as the Hispanic population increases), its current economic value as a crop is much less than it might be, if produced for the manufacture of products used in treating hypoglycemia, diabetes, high blood cholesterol and obesity [6].

The fruit is rich in flavonoids, polyphenols and betalains that act as antioxidant compounds and neutralize free radicals before they cause healthy cells to mutate. Free radicals are partially responsible for the oxidation of neural cells that lead to various diseases. Polyphenolic compounds also, have been linked to increased cognitive activity [5].On the other hand, betalains are water-soluble nitrogen-containing pigments that are responsible for the bright red or yellow color of fruits, flowers, roots and leaves of plants. Prickly pear contains, particularly, betacyanins in the purple variety and betaxanthins in the orange variety [7].

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MATERIALS AND METHODS

The prickly pear fruits were obtained from Greece and they were grown on the most suitable soil. Only natural fertilization was employed. Special attention was paid to ensure absence of any chemical fertilizers, pesticides, insecticides, fungicides, or herbicides.

The harvest of the plant (leaf together with fruit) was conducted according to a strict protocol and collection rules. The collected parts included unripe fruits, leaves and ripe fruits (in their early ripening stage-not fully matured). The collection took place during late mornings of August; the most suitable hour for its collection is late in the morning, to avoid moisture. Only the completely dry and clean (of parasites, or illness) fruits of the plant were collected. Meticulous cleaning of all kinds of impurities (organic or inorganic) followed. Only well-ventilated packages (perforated bags, baskets of reeds, boxes full of holes etc.) were used in order to rule out the growth of harmful enzymes and fungi. Furthermore, the longest length of transport of the plant never exceeded 48 hours.

Drying process.

The leaves at the beginning went through a process called stabilization, whereby the leaves were steamed in 60% pure ethanol. Subsequently, the leaves had the thorns removed with special tools, and then were put in a well ventilated place in the shade for drying. Drying usually lasted 7-15 days (depending on the weather).

The thorns of the fruits were removed with a special brush; the fruits were then peeled and the peel removed was also placed for drying under the same conditions as the leaves.

Fruit seeds were separated from the unripen fruits. They were then subjected to a stabilization process. They were subsequently placed in the sun to dry 1–2 days prior to being used for the extraction. The stabilization process for natural entities which are intended to be used in an extraction process after being dried, involves the following: placement of the respective compounds in a form of a very thin layer on the top of a sieve tray; a very fine droplet spraying of the herbal substance with pure ethanol follows, so that the whole exterior surface of it is wetted. The ethanol is then left to dry. This process is the most suitable natural method of elimination of any type of fungi or bacteria which can affect the herbal entities and prevent its decay until it is taken for the extraction

Extraction.

Three extractions were made, one of the leaves together with the peel of the fruits, one of the ripe fruits containing their seeds and one of the seeds of the unripen fruits.

The extraction process involved maceration followed by percolation

A. Maceration

The herbal substance had first been crushed to form a coarse powder. The resulting coarse powder (of a size appox5 mm) was mixed with the solvent, which was usually water, pure alcohol or a mixture of pure alcohol & water in a volume proportion 50:50. It was allowed to soak for 1day. During the day the mixture was shaken regularly & finally formed a pulp like extract.

B. Percolation

The aforementioned pulp containing the herbal substance, after the maceration, was placed in a special apparatus for 3 days with enough pure solvent (ethanol) to completely cover the herbal substance. Subsequently, it was placed inside the extractor a specific gravity with specific diameter and kg, according to specifications and it was kept so for at least 24 hours. The extract was filtered or it was left to rest until it was clear.

Fig.1. *Opuntia ficus indica* extracts after maceration and percolation process; from left to right: extracts from unripe fruit seeds (in conical flask), from leaves and fruit peels (large beaker with light colored liquid), and from ripe fruits containing their seeds (smaller size beaker), respectively.

HPLC analysis.

The samples were analyzed using a Luna, 4.6×150 mm, 5 m particle size, 100 °A pore size column. A 40:60 acetonitrile:water solution was used for 14 min, as an isocratic mobile phase at 0.5 ml/min flow rate and 30 ºC. The injection volume was 20 microliter and detection was through a UV detector set at 280 nm.

Well defined in a strict protocol quality controls were performed during the procedure and at the end of the process. Those protocols involve a number of analytical methods which have been developed in our laboratory through over 20 years of experience; they were based on traditional analytical methods of Galenea and French pharmacology but PANAX has advanced and specialized their applicability and reliability. The final quality control of the extract included determination of alcoholic degree and degree of acidity (pH) of the extracts.

RESULTS AND DISCUSSION

In this work the treatment of prickly pear employed dried fruits to form powder and liquid extracts. They lead to production of pharmaceutical products such as capsules, tablets, drops, powder and elixirs. The most important feature of this production is that all materials and products are non-toxic, natural and friendly to the environment.

The results from the HPLC analysis from the three extractions are observed at the figures 2,3 and 4.

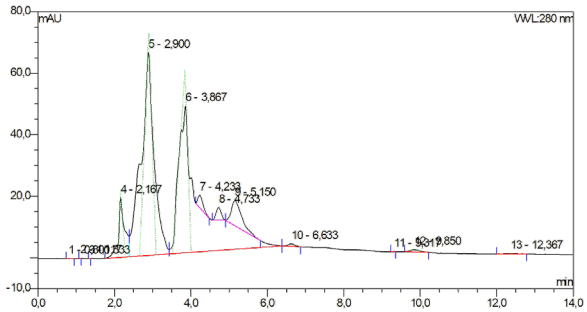

Fig.2. HPLC absorption diagram at 280 nm obtained from ethanolic leaves and fruit-peels extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units.

As can be seen in Figure 1, a number of compounds are eluted. Two major peaks indicate the key compounds of the extract from the leaves and peels of the fruit. The two main peaks appear at 2.9 min. and at 3.867 min retention time.

Figure 2 also shows two main substances to be obtained from the extract of the ripe fruits with their seeds. The retention time of those compound areslightly different namely, the two compounds are eluted at 2.867 and 3.950 min, respectively.

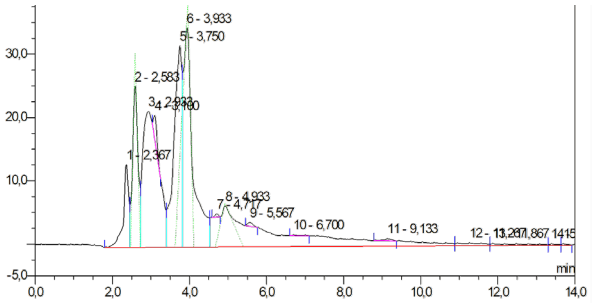

Fig.3. HPLC absorption diagram at 280 nm, obtained from ethanolic ripe fruits with seeds (prickly pear) extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units

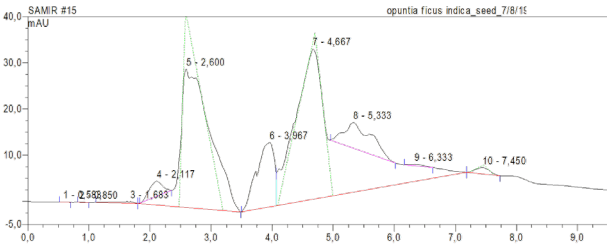

Fig.4. HPLC absorption diagram at 280 nm, obtained from ethanolic unripen fruit seeds extract from the *Opuntia ficus indica.* Absica shows retention time in min and ordinate arbitrary absorption units

Finally, figure 4 shows two major peaks, indicating again the existence of two main substances to be obtained from the extract of the unripen fruit seeds. The retention time of those compounds are significantly different that the other two. The main substances of the extract from the seeds give two main peaks at 2.6 min and at 4.667 min.

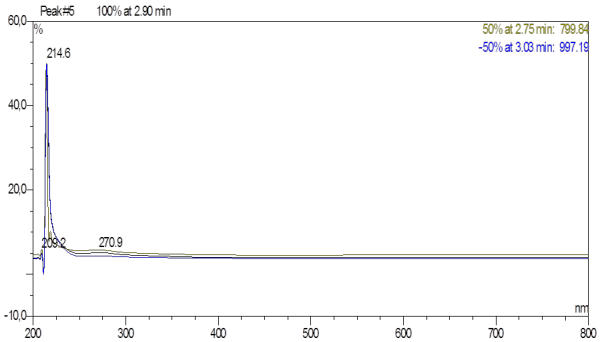

Fig. 5. Typical UV spectrum of the compound of the compound with retention time of 2.9 min of an extract from the leaves and the fruit peel of *Opuntia ficus indica.*

Figures 5 and 6 show the UV spectra which correspond to the two major peaks taken from a typical extract of the leaves and the the peel of the fruit. Both demonstrate three absorption peaks at 209, 215.2 and 270.2 nm indicating that they correspond to the same compound. Further

chemical analyses to identify composition of those extracts are currently underway.

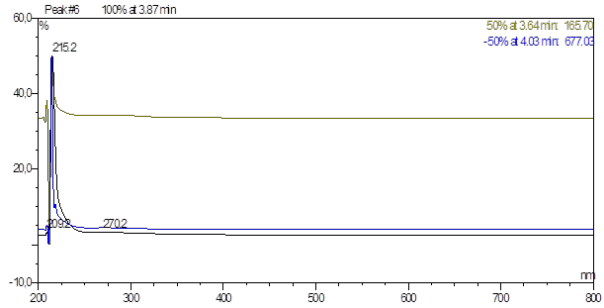

Fig. 6. Typical UV spectrum of the compound of the compound with retention time of 3.867 min of an extract from the leaves and the fruit peel of *Opuntia ficus indica*.

The measurement of acidity (pH) of the final extracts is shown in Table1. As can be seen, the leaf and fruit peel extracts are the most acidic of the three extracts obtained in the current study.

Table.1. Results from the measurement of acidity (pH)

Extract	pH
Leaf and peel extract	5,1
Ripe fruit with seeds extract	5,4
Unripen fruit seed extract	5,8

The extracts obtained via the above described methodology can be used either as individual medicinal formulations or in combination with other natural extracts to reestablish the healthy natural functions of the body.

Prickly pear is also suitable for expanding feedstock production into semi-arid marginal lands, as it represent highly water-use efficient bio-energy crop. That type of feedstock has garnered interest because of its high water- and fertilizer-use efficiency and not competing with major food crops or conventional bio-fuel feedstock [8]. Furthermore, the employed mild extraction procedure is such that key components useful for biofuel production remain unaffected. As such, the residual biomass can be further valorized via the production of chemicals, fertilizers or biofuels.

CONCLUSION

Over the last year there has been an abundance of scientific papers on cactus pear as a source of bioactive compounds for nutrition, health and disease, underlining the interest in the numerous properties (both its bioactivity and coloring potential) of this plant species, well adapted to extreme growing conditions in arid and semi-arid zones [7].

In conclusion, the uses of prickly pear can be summarized into the following:

- ✓ Food and beverage industry
- ✓ Feed industry
- ✓ Drug industry
- ✓ Cosmetics industry
- ✓ Food supplements industry
- ✓ Manufacture of natural additives
- ✓ Energy sector
- ✓ Fertilizers

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