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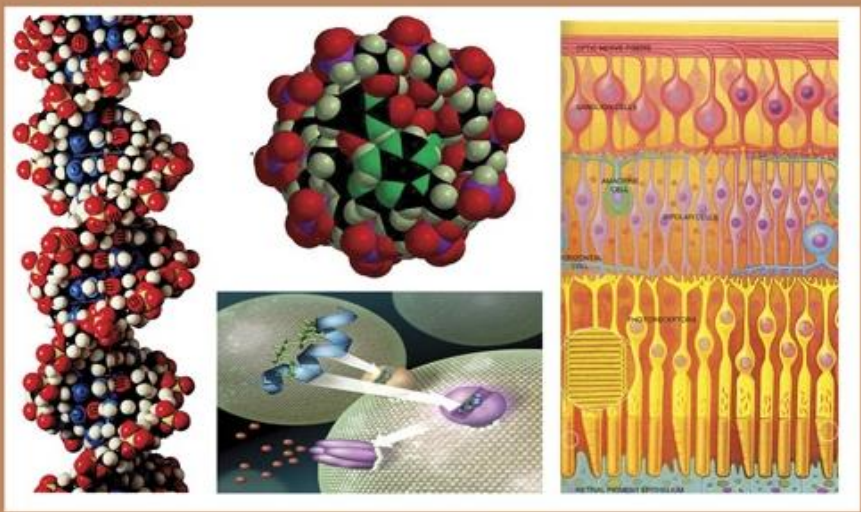

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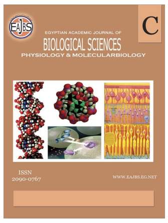

Flavonoids *crataegus oxyacantha* Bioassay *in Vitro* (35%ethanol 65% water) with Plant Bionanosensor

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ABSTRACT

Hawthorn extract has a significant amount of flavonoid and is of high importance due to the antioxidant property. Bioassay is a biological testing procedure for estimating the concentration of a substance. There are various methods for measuring antioxidant biological compounds. Qualitative and quantitative techniques of polyphenols measurement include chromatography, HPLC and GC-MS. These techniques are expensive and time-consuming so the development of biosensors can overcome these limitations. In order to prevent damage by free radicals, the body has a defense system of antioxidants. Plant bionanosensor is a certain type and novel approach of biosensors that has been fabricated by this author for the first time. The study was used to determine two types of flavonoids concentrations in vitro (35%ethanol 65% water) in hawthorn extract through rotation with three replications by sas9.1 software. The different levels of flavonoid rotation are significant with a probability of 99%. So far, no report was made about such a plant bionanosensor at international level from other researchers.

INTRODUCTION

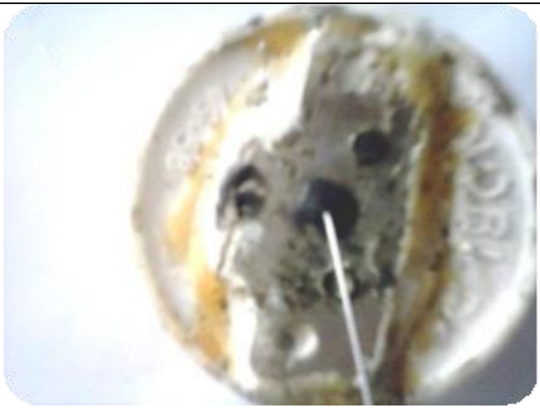
Micro-organisms, tissues, cells, organelles, enzymes, peptides and DNA are used to identify the target substance called a specific or nonspecific biosensor. bioassay is a biological testing procedure for estimating the concentration of a substance in a formulated product or bulk material (Moradi *et al.*, 2018). The *sensitive biological element*, e.g. tissue, microorganisms, organelles, cell receptors, enzymes, antibodies, nucleic acids, etc., is a biologically derived material or biomimetic component that interacts, binds, or recognizes with the analyte under study. The biologically sensitive elements can also be created by biological engineering. The *transducer* or the *detector element*, which transforms one signal into another one, works in a physicochemical way: optical, piezoelectric, electrochemical, electrochemiluminescence etc., resulting from the interaction of the analyte with the biological element, to easily measure and quantify. The biosensor reader device with the associated electronics or signal processors that are primarily responsible for the display of the results in a user-friendly way *(Cavalcanti, et al., 2008).* Hawthorn extract has a significant amount of flavonoid and is of high importance due to the antioxidant property. Flavonoids have two groups of anthocyanins and antoxantine.

Anthocyanin has pigments and antoxantine is colorless ((Kin and Young, 1999). Due to the importance of flavonoids as antioxidants and expensive and time-consuming methods, bioassay of flavonoids via biosensor is growing. Plant bionanosensor is a found approach, So far, two reports were made on plant biosensor by this paper's author (Moradi et al., 2018). He began his research of plant biosensors with patent No. 26299 in Iran in 1999 with the discovery of rotation organ (Moradi, 1999).Then, in 2000, he was chosen in Khwarizmi Festival thanks to the invention named intelligent organ and could gain third place (discovery of smart organ, 2000). He recorded the plant sensor No. 332/421 in Iran's scientific research and industrial organization in 2004. Foundation of the memory material was reported in Iran in 2005 ((Moradi, 2000) and a type of bioreactor sensor was built (Moradi *et al.,* 2003). From the intelligent organ, a plant engine generating bioenergy was created under Biotechnology Master's thesis at Tehran University with the participation of Sharif University (Moradi, 2005) and was patented in Iran (Moradi, 2005). Moreover Mobile safety sensor (Moradi, 2010), plant anchor cells as storage energy (Moradi, 2005) moisture signal sensor (Moradi, 2005) damp-proof Nanosensor of drugs and materials (Moradi, 2006) industry and materials micro-sensor (Moradi, 2005) were patented in Iran and one file named nanomotor was recorded in US (Moradi, 2009). Foundation of plant sensors using discovering intelligent organ was accepted at the University of Arkansas of US (Moradi, 2009) and foundation of cellulosic rotating Nanocomposites, including NanoBiotech were accepted at Switzerland (Moradi, 2009) international nanotechnology conferences. The mobile safety sensor obtained the bronze medal in the Olympics (Moradi, 2011) and was verified by the three-level of Iran's National Elite Foundation (Moradi, 2011). Biosensors are based on identifying the target material with high specific detection (18 Mello and Kubota, 2002) as follows a. bio-recognition elements: in which enzymes, antibodies, a part of DNA, peptides, and even a tissue of microorganism are used (Gooding, 2006).b. Amperometric biosensors that act based on oxidation and electrode reduction coated with enzymes (Gooding, 2006). Absorption biosensors bind to a molecule that is to be determined. The electrode with coated enzyme is one of them. Carbon paste electrodes are widely used initially by Adams (Mailley *et al.,* 2004). Carbon paste electrodes are simple and cheap (Ghobadi *et al.,* 1996; Bolado *et al.,* 2007). c.electrochemical analysis of polyphenolic compounds: quality and quantity of polyphenols are done by spectrometry or HPLC that are expensive and time-consuming. Graphite powder compound with non-electrolytic paste is inexpensive and easy to use. For more polyphenolic compounds, antioxidants containing phenolic groups are commonly used, which are based on electrochemical biosensors activity. They are a. polyphenol in the oil extraction (Capannesi *et al.*, 2000), b. red polyphenol compounds (Gomes *et al.*, 2004) c. polyphenols of various teas (Campanella *et al.*, 2004; Campanella *et al.*, 2005), d. white and red polyphenols (Campanella *et al.*, 2004; Campanella *et al.*, 2005) and a type of biosensor with Laccase enzyme for measuring the red polyphenol compounds. Laccase biosensor directly causes the oxidation of phenolic compounds (Freire *et al.*, 2001; Freire *et al.*, 2003). There are various methods for measuring antioxidant biological compounds. Qualitative and quantitative techniques of polyphenols measurement include chromatography such as HPLC and GC-MS. These techniques are expensive and time-consuming so the development of biosensors can overcome these limitations. In order to prevent damage by free radicals, the body has a defense system of antioxidants (Antolovich *et al.*, 2002). Plant bionanosensor is a certain type and novel approach of biosensors that has been fabricated by this author for invention plant nano-sensor. The study was used to determine two types of flavonoids concentrations *in vitro* (35%ethanol 65% water) in hawthorn extract through rotation. So far, no report was made about such a plant biosensor at international level from other researchers.

MATERIALS AND METHODS

Plant Nano-structure used in plant bionanosensor is the rotating Nano-structures as storage (i.e., it can turn to its previous memory such as memory metal) that has been fabricated by the author of this paper. according to the following figure, plant bionanosensor has a graded plane divided by 310 sections, in which the amount of rotation could be read by a hand connected to a plant nano-structure (Figs. 1 & 2). In the bottom of the bionanosensor, the test site of the extract flavonoids can be seen (Figure). Using insulin syringe, a drop of treatment inserted at the test site and the test site was placed on the paper to read the degree of each treatment 1-1.25 mg, 0.625-0.5 mg and 0 mg of flavonoid on hyperosoide/1 ml of hawthorn (crataegus oxyacantha) in vitro (ethanol 35% and65%water) was purchased by pharmaceutical company of Iran Daru.

With regard to injection of 1.100 cc, the treatments were divided into 100 and the effect of treatments was analyzed by 0.0125- 0.01 mg, 0.0625-0.005 mg and 0 mg flavonoid in 1.100 cc as a completely randomized experiment with three iterations by software sas9.1. a comparison of average rotation was performed with LSD test at 1% level.

	
Fig 1.Plant bionanosensor	Fig 2. The site of flavonoids test

RESULTS AND DISCUSSION

According to Table1, at least two of the treatments are statistically different from each other at 1% in order to decide which one of the treatments has a significant difference with other treatments. LSD method was used to compare means.

Table 1. Analysis of variance of the treatments rotation of flavonoids extract

Source of changes	Degree of freedom	Mean Square F**
Treatment	2	8633.33
Error	6	63.88

R2=0.97 c.v= 3.89 **sig=1%

Table 2 shows that the A treatment has the greatest effect on rotational memory that is related to control and showed the greatest rotation by the greatest uptake by plant bionanosensor and the D treatment has the lowest effect on the rotational memory because it has the highest concentration. Therefore, it has low absorption and low rotation.

Table 2. Comparison of the mean treatments of Flavonoid concentrations

Flavonoid mg as hyperosoide in vitro(35% ethanol and 65%water) of crataegus oxyacantha extract	The average degree of rotational memory	Rank
0 (control)	265.00	A
0.005-0.00625	188.33	B
0.01-0.125	161.66	C

According to the above tables, the plant bionanosensor is capable to detect hawthorn extract with a probability of 99%. The higher the flavonoid concentration, the lower the degree of rotation sensors will be. To examine the cause of rotation through an electron microscope manufactured by Zeiss German Factory, model 60A at Tehran University by SEM of plant sensor used in plant biosensor, a scan was run. The following picture is one of the images obtained.

The Figure 3 shows that the lower part of the picture magnified 1,500 times, in which nanoparticles less than the mean diameter of a nanometer or nano-holes can be seen that contribute to its rotation. The Nano-structure longitudinal section shows that nano-holes and nanoparticles with absorption treatments convert the absorption treatment energy into rotation with the help of specific structural features. Plant bionanosensor advantage includes: a. it is inexpensive, easy to use and portable, compared to chromatography, spectroscopy techniques and other biosensors. b. It has been the result of research and invention, since 1999

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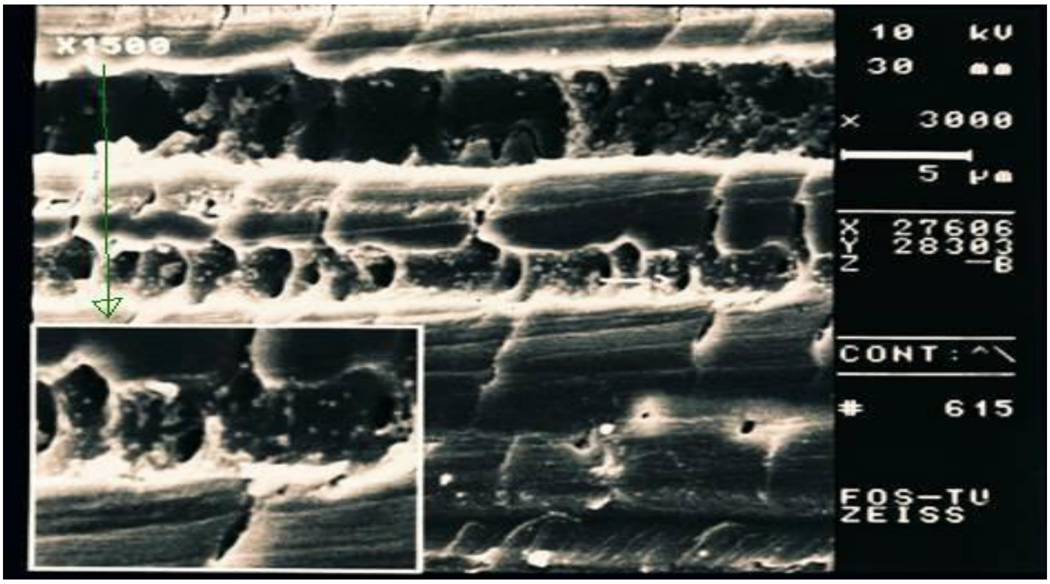

Fig. 3. Longitudinal section- electron microscopic image of plant nanostructure by SEM

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