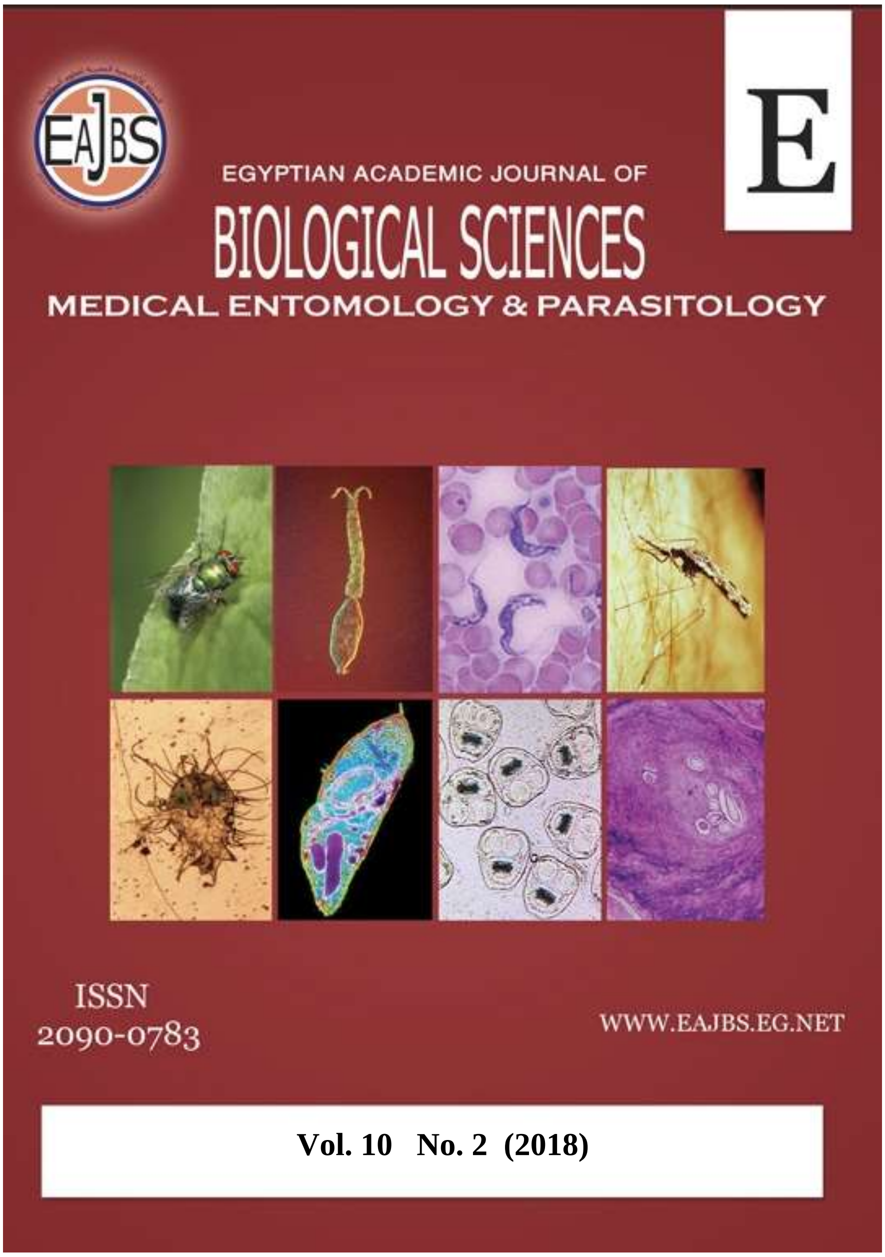

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The Qualitative Bioassay of vitamins C and B12 antioxidants by Plant bionanosensor

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ABSTRACT

If the micro-organisms, tissues, cells, enzymes, DNA, or peptide used to identify the target, the process is known as a biosensor. HPLC and UV radiation are used for Vitamins measurement which is time-consuming and expensive. So, biosensors can remove the restrictions. In this study, for the first time, the amount of water-soluble vitamins by plant bionanosensor that is a new approach was became to a visible rotation, and its amount can be detected through this rotation. For the detection of vitamin B12 and vitamin C antioxidants in the form of injection ampoules, the rotational effect of them was analyzed by plant bionanosensor in a completely randomized design with three replications by sas9.1 software. 10.5It was determined that plant bionanosensor capability of detecting vitamin B12 and vitamin C through the rotation is likely to be 99%. Plant bionanosensor converts the existence of these two types of vitamins to a meaningful rotation, so its rotation is a criterion for measuring.

INTRODUCTION

Consumption of beverages that contain vitamins such as vitamin C and vitamin B12 are increasing and the consumption of drinks containing vitamins had got 4388 million liters per year by 2011 (Sharpless, Katherine E, *et al.* 2000). Vitamins Detection in beverages is difficult (Klimes, J,et al 2005) and analysis of such beverages faces challenges, because of the water-soluble and fat-soluble vitamins. Water-soluble vitamins are recognized by RP-HPLC and fat-soluble vitamins by HPLC (Margolis, Sam A *et al.* 1996). UV radiation is also used to measure some of the vitamins such as C and B12. The necessary wavelength is required to optimize the sensitivity. The single method is used for both water-soluble vitamins and fat-soluble vitamins, in which a silica layer and an amide ligand attached to the silica are applied for PH (1.5-10), a hundred percent aqueous mobile phase is used at the beginning of work and a hundred percent organic solvent mobile phase at the end. This method is a fast and low-cost solution for recognizing water- soluble and fat-soluble vitamins of an injection (Zegler, Jennifer,2008). According to the assessment methods, standardize of production is necessary (www.cfsan.fda.gov).The student Author of the paper has started his research in the field of plant biosensors with the discovery of a rotating organ and by patent No. 26299 in 1999 in Iran (Moradi ,F,1999). Then in 2000, by presenting the innovation to Qwarizmi Festival, he was chosen for the discovery of intelligent organ and succeeds to win the third place (Moradi ,F,2000). In 2004, he registered a kind of plant sensor (No.332/421) at Iran scientific and industrial research organization. In 2005, he established the memorial plant material in Iran (Morad ,F,2005) and built a type of bioreactor sensor (Moradi ,F, et al,2003). He built an intelligent organ of plant engine which generates bio-energy as his biotechnology senior thesis at Tehran University and in cooperates with Sharif University (Moradi ,F,2005). This engine is patented in Iran (Morad ,F,2005). Also plant anchor cells as a source of memorial energy (Moradi ,F,2005), moisture signal sensor (Moradi ,F,2005),dehumidifier Nano sensor of drugs and materials (Moradi ,F,2005), mobile safety sensor (Moradi ,F,2010) materials and industry micro-sensor is patented (Moradi ,F,2005) and a file called nanomotor which is registered in America (Moradi ,F,2006).At international nanotechnology conferences, such as NanoBiotech Switzerland, the establishment of cellulosic spinning Nano-composites was accepted (Moradi ,F,2009). At the University of Arkansas America, the establishment of plant sensors by the discovery of intelligent organ was accepted (Moradi ,F,2009) and mobile safety sensor succeeded to win the Olympiad bronze Medal (Moradi ,F,2011) and 3th level grant approval of Iran National Elites Foundation (Moradi ,F,2011).

Biosensors with high specific diagnosis are based on the identification of the target material (Mello, L.D.,2002), including: 1-biological recognition elements, enzyme (antibody), some part of DNA, and even peptides of a living creature have been used in this kind of biosensors (Gooding, J.J. 2006.). 2- Amperometric biosensors which operate based on oxidation and electrode revival that is coated with enzymes (Vastarella, W. 2001).Absorptive biosensors stick to the molecular that is supposed to be determined. The electrode which is coated with enzymes is one of them. 3- The sticky carbon electrodes had been initiated by Adams, and nowadays are used widely (Mailley, P.,*et al*,2004). The sticky carbon electrodes are simple and cheap (Ghobadi, S.,1996, and Bolado, P.F.,*et al* 2007). 4- The electrochemical analysis of polyphenolic compounds which is a kind of biosensor with Laccase enzyme has been developed for measuring the polyphenolic compounds in red (Antolovich, M.,*et al*,2002 and Katalinic, V.,*et al*,2009). Studies show that consumption of fruits and vegetables reduces the risk of cardiovascular disease, cancer and degenerative processes in the body tissues (Wolfe ,K.2003, Sanchez-Moreno C.2003). The biological effects of antioxidant compounds such as vitamin Chelpto prevent the destruction of tissues by free radicals (Toit, R,2001, Proteggente, AR.2003) the amount of vitamin C is a key indicator for defining the commercial value of fruits and vegetables (Saari ,NB.,1999).The qualitative and quantitative techniques for measuring antioxidants are based on chromatography separation such as HPLC and GC-MS, but these techniques are expensive and time-consuming. In order to prevent the damages of free radicals, human body has antioxidant defense system (Katalinic, V.,*et al*,2006). In this regard, antioxidants such as vitamin C and vitamin B12 play a role, so they need to be measured and the chemical titration is a simple method to measure the amount of vitamin C (Arya SP. N,2000). There are various methods for measuring an antioxidant capacity of the biological compounds. In general, 3 chemical methods are used to evaluate the antioxidant features:

1-DPPH (2, 2 diphenyl-1-picrylhydrazyl free radical): The DPPH stoke is used to determine the deterrence feature of free radicals of pure or herbal substances (Arya SP,N, et al 2008).

2-ABTS test (2, 2 Azinobis3-ethylbenzothiazoline-6-sulfonicacid): this is performed according to the Arnao et al. method (Arano, M,2001).

3-The linoleic acid model: the linoleic acid is used in this method (Ingold, K.U.1968). The plant bionanosensor is a specific type of biosensors and has been made by the Faramarz Moradi for the first time. The plant bionanosensor is made from plant nanostructure and in this study is used to distinguish two types of antioxidants, vitamin B12 and vitamin C in the form of injectable ampoules. So far no reports about such a plant bionanosensor have been presented by other researchers at the international level.

MATERIALS AND METHODS

The plant bio Nano sensor is made by the Faramarz Moradi, this sensor is made from plant material which has memorial rotary motion (scilicet, it is able to turn back to its previous turn or previous). As it can be seen in the following picture, plant bionanosensor has a graded screen which is divided to 310 parts; the spin amount is readable by its hand which is connected to the nanostructure (Fig 1and fig 4).

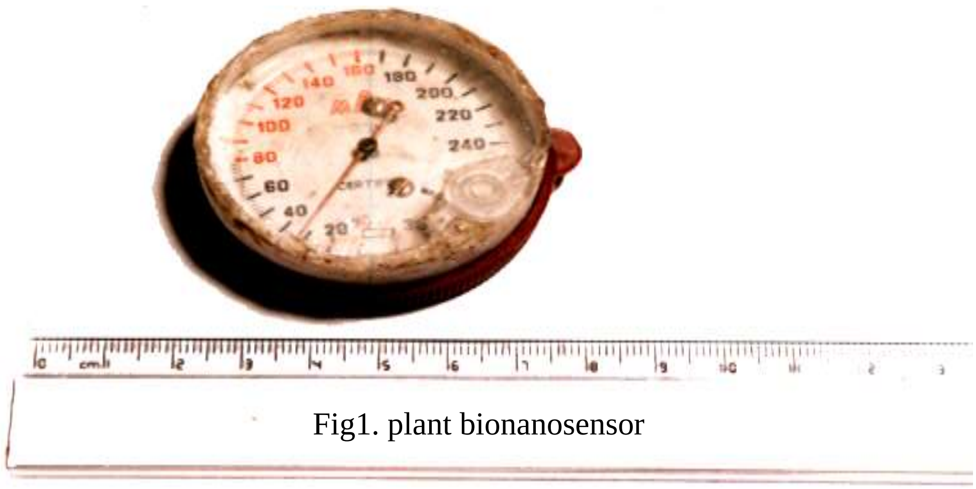

Fig1. plant bionanosensor

Vitamin B12 and vitamin C in the form of injectable ampoules was purchased from Iran Daroo pharmaceutical. There is a treatment test site in the bottom of the plant bioNano sensor (Fig 2), a drop of vitamin B12 and vitamin C injected by using 2 cc syringe at the test site (Fig 3).

Fig 2

Fig 3

In order to read a degree of each treatment, the test site was placed on a paper.

The rotation effect of the three types of treatment, the distilled water (as a control), and vitamins B12 and C in the form of **injection ampoules manufactured by Tehran** Daroo pharmacy company were analyzed in a completely randomized design by software sas9.1 with three repetitions.

RESULTS AND DISCUSSIONS

Results of rotation bioassay are presented in table 1.

Table1.The degree of plant bionanosensor rotation

Treatment	Distilled water rotation	Vitamin B12 rotation	Vitamin C rotation
Repetition	200	170	70
	220	180	80
	210	150	90

Table2. Analysis of the rotation variance of Vitamins B and C treatments and distilled water

Resource of changes	Degree of freedom	Sum of Squares	Mean squares ** F
Treatment	2	26289	91 13144.45
Error	6	867	144

Significant at 1%, CV=7.8

According to Table 2, at least two groups of these 3 treatments are statistically different at the level of 1%. To determine which one of these treatments have a significant difference with other ones, the averages were compared by Duncan method.

Table 3. Comparison of the mean treatments (vitamin B12, vitamin C and distilled water**)** ethanol (70% ethanol and 30% water

Treatment	The average means of rotation	rank
Distilled water rotation	210	A
Vitamin B12 rotation	166	B
Vitamin C rotation	80	C

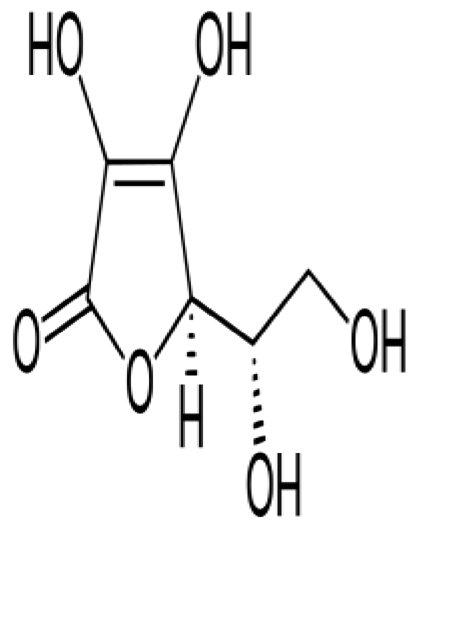

Fig 4. Vitamin C

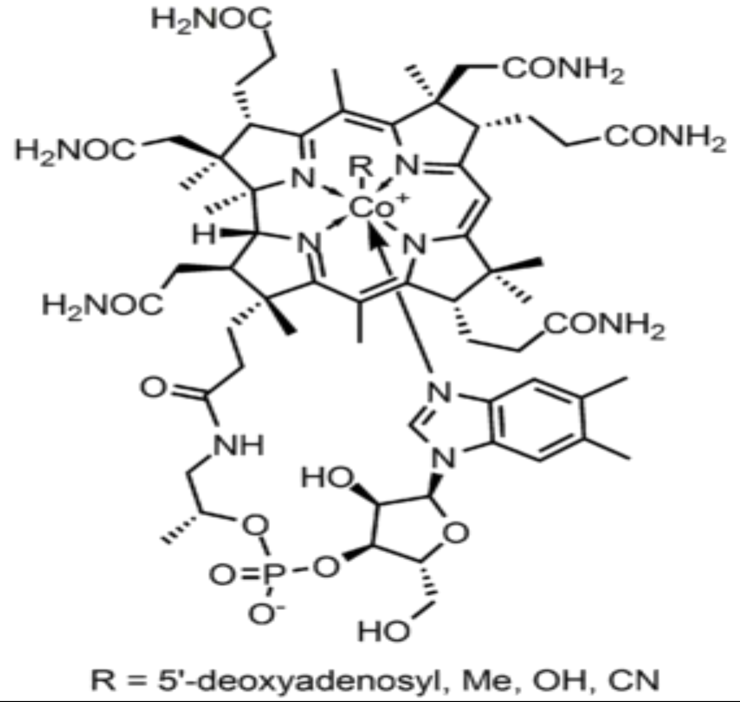

Fig 5. Vitamin B12

Based on the tables above, by the existence of distilled water as a control, plant bionanosensor capability of detecting vitamin B12 and vitamin C antioxidants from each other is likely to be 99%. Distilled water showed the highest degree of rotation and vitamin C showed a lower degree because distilled water absorption is higher than other treatments. Based on the SEM method, the plant sample used in bio nanosensor was scanned by an electron microscope manufactured by Zeiss Germany, model 60A, at Tehran University study. The picture below is one of the obtained images.

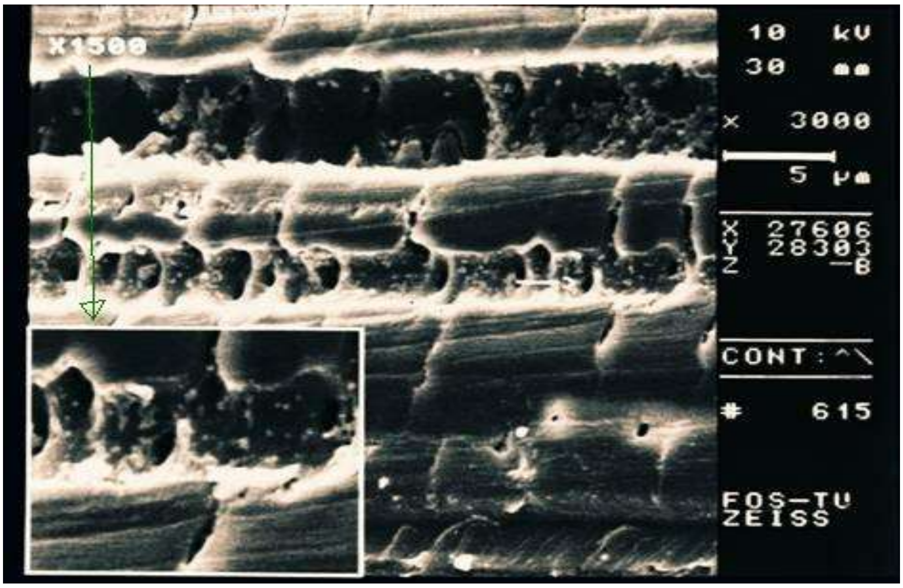

Fig7.Longitudinal-section, the image of electron microscope of plant sensors based on SEM method

The picture shows that the lower part of the picture has got bigger 1500 times, and some Nano structure and Nanoholes are seen which their diameter is less than normal particles and Nano-holes, they are involved in the turn.

Given that vitamin C is considered as an indicator of the commercial value of fruits and vegetables and vitamin C and vitamin B12 are considered as antioxidants, recognizing of their amounts is necessary, so the advantages of using plant bionanosensor include:

1. It is cheaper than chromatography and spectroscopy methods.
2. It is the result of research work, since 1999.
3. The method is simple and its ease of transport makes it applicable in any conditions.
4. Unlike other methods, it did not use any electrical energy source, because of vitamin B12 and vitamin C act as the biosensor energy source.
5. If registered internationally, it can be commercialized in the international level.
6. If get optimized, it would be possible to study the plants secondary metabolites and other physiological features directly and through intact leaves.
7. The method is simple, cheap and easy to use.
8. It is high speed.

Suggestions:

If a biosensor would make by free radicals, it can recognize all the antioxidants. So far, such biosensors have not been reported. Current biosensors are made based on enzymes and reactive or broking material, which limits the performance.

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