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The flavonoids, vitamin C and B12 have a prognosis on Covde-19 infections and detect by plant bionanosensor *in vitro*

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Abstract
Plant secondary metabolites are an important source of new drugs, especially flavonoids, were shown to have antioxidant, antiviral, antibacterial, anticancer. . Antioxidant compounds have the potential to improve the prognosis of COVID-19 infections. The antiviral effect of flavonoids, which are secondary plant phenolics, as well as other antioxidants has been explored for their effect on SARS-CoV-2. There are various methods for measuring biological compounds. Qualitative and quantitative techniques of polyphenols measurement include chromatography such as HPLC and GC-MS. These techniques are expensive and time-consuming .In this study, for the first time, the amount of vitamins of vitamin B12, vitamin C and Hawthorn extract flavonoids by plant biosensor that is a new approach was become to a visible rotation, and its amount can be detected through this rotation. For the detection of vitamin B12, vitamin C and Hawthorn extract flavonoids the rotations of them were analyzed by plant bionanosensor in a completely randomized design with three replications by sas9.1 software. It was determined that plant bionanosensor capability of detecting vitamin B12 , vitamin C and flavonoids through the rotation is likely to be 99%

Keywords: plant bionanosensor, COVID-19, vitamin B12 and vitamin C.

Introduction

Plant secondary metabolites are an important source of new drugs, especially flavonoids, were shown to have antioxidant, antiviral, antibacterial, anticancer (clere et al., 2011; Pan et al., 2010). a recent study showed that methyl cobalamin supplements have the potential to reduce COVID-19-related organ damage and symptoms. Given that, it is plausible that symptoms of vitamin B12 deficiency are close to COVID-19 infection such as elevated oxidative stress (Sabry, et al., 2020). The use of antioxidants in Chinese herbal medicine has been widely studied, with some antiviral effects reported to occur when using these agents following infection by SARS-CoV-2. The antiviral effect of flavonoids, which are secondary plant phenolics, as well as other antioxidants has been explored for their effect on SARS-CoV-2. These antioxidants have been found to have a positive effect with different antioxidants working in various ways. An example of this is nigellone, which was found to bind parts of the SARS-CoV-2 virus such as the S protein, as well as block inflammatory markers such as interleukin-1 (IL-1) and IL-6. Other effects of antioxidants in the treatment of SARS-CoV-2 include, but are not limited to, the inhibition of viral replication as well as preventing the entry of the virus into the host cell. As there are no specific antiviral drugs available for SARS-CoV-2, adjuvant therapies can be useful in treating COVID-19. Antioxidant compounds also have the potential to improve the prognosis of COVID-19 infections. With the ability to reduce inflammation, the researchers discuss the potential utility of incorporating dietary antioxidants into a COVID-19 patient's treatment plan. Some of the specific dietary antioxidants that could have a therapeutic effect include vitamins A, C, D, B6, and B12, folate, zinc, iron, copper, and selenium, as each of these micro nutrients have a role in the immune response. These vitamins, particularly vitamin C, have also been shown to have an effect on COVID-19 infections .Other neurological protectants can also help the recovery after brain injuries due to ischemia. Neurological management is an essential component of COVID-19 treatment. To this end, the use of antioxidants to reduce the inflammatory response while simultaneously working as a neuro-protectant could support their use as adjuvants in treating SARS-CoV-2, especially as new variants of SARS-CoV-2 continue to emerge around the world (cadenas, et al,.2021). In this regard, antioxidants such as vitamin C, vitamin B12 and crataegus oxyacantha (Hawthorn) extract flavonoids play a role, so they need to be bioassy and plant bionanosensor is a simple method. This study aimed at developing

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biosensors for key intermediates of the vitamin C vitamin B12 and Flovenoids against Covid19 effects by development of plant bionanosensor based on rotation to vitamin C, vitamin B12 and crataegus oxyacantha (Hawthorn) extract flavonoids Bioassy. . There are various methods for measuring 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, human body has a antioxidant defense system (Katalinic, V .,*et al*,2006). Some plants have antioxidant properties, especially biological flavonoids are considered as neutralizing free radicals due to antioxidant, natural origin and effective ability of activity. The flavonoids, are used to eliminate free radicals. Plant bionanosensor is a certain type and new approach of biosensors that has been used by the Faramarz Moradi as inventor of this paper. This study was used to determine concentrations of flavonoids in hawthorn extract and vitamins through rotation. So far, no report was made about such a plant bionanosensor at international level from other researchers (Moradi, F et al, .2018)

Consumption of beverages that contain vitamins such as vitamin C and vitamin B12 are increasing and the consumption of drinks containing vitamins had got4388 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. .Faramarz.Moradi has started this research in the field of plant biosensors in 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 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 and built a type of bioreactor sensor, mobile safety sensor is patented and a file called nanomotor which is registered in America .At international nanotechnology conferences, such as NanoBiotech Switzerland, the establishment of cellulosic spinning Nano-composites was accepted. At the University of Arkansas America, the establishment of plant sensors by the discovery of intelligent organ was accepted , mobile safety sensor succeeded to win the Olympiad bronze Medal and 3th level grant approval of Iran National Elites Foundation (Moradi ,f et al,. 2018, Moradi,f,..2019) 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 issupposed to be determined. The electrode which is coated with enzymes is one of them. 3- The sticky carbon electrodes had been initially 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 Studies show that consumption of fruits and vegetables reduces the risk of cardiovascular disease, cancer and degenerative processes in the body tissues (Wolf, K.2003, Sanchez-Moreno C.2003). The biological effects of antioxidant compounds prevent the destruction of tissues by free radicals (Toit,R,2001, Proteggente, AR.2003).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. The plant bionanosensor is made from plant nanostructure and is used to distinguish two types of antioxidants, vitamin B12 and vitamin C (70% ethanol and 30% water) 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. (Table 1 and Table 2 Moradi, F et al., 2018).

Table1. 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 1, 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

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by Duncan method.

Table 2. 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

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

Materials and Methods

The plant bio Nano sensor is made by the author of the paper, this sensor is made from plant material which has memorial rotary motion (it is able to turn back to its pervious turn). As it can be seen in the following picture, plant bionanosensor (fig 1) has a graded screen which is divided to 310 parts; the spin amount is readable by its hand which is connected to the nanostructure (Moradi, f et al, 2018).

Fig1. Plant bionanosensor (Moradi, f, et al, 2018). Fig 2(Moradi, f, et al, 2018, Moradi, f, 2019).

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There is a treatment test site in the bottom of the plant bioNano sensor (Fig 2), a drop of treatments 02- 0.025 mg flavonoid on hyperosoide, 1 mg vitamin C , 5 mg vitamin B12 and 0 mg flavonoid in 1.100 cc injected by using syringe at the test site . In order to reading a degree of each treatment, the test site was placed on a paper. 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. 2-2.5 mg flavonoids on hyperosoide/1 ml of hawthorn extract 100 mg vitamin C, 500 mg vitamin B12 and distilled water(as a control), purchased from pharmaceutical company of Iran Daru. With regard to injection of 1.100 cc, the treatments were divided into 100 and the rotation of treatments 02- 0.025 mg flavonoid on hyperosoide, 1 mg vitamin C, 5 mg vitamin B12 and 0 mg flavonoid in 1.100 cc were analyzed by as a completely randomized experiment with three replications by software sas9.1. Comparison of average rotation was performed with LSD test at 1% leve

Results and discussions

Table3. Analysis of the rotation variance of Vitamin B ,vitamin C , *crataegus oxyacantha* extract(flavonoids) and distilled water treatments

Resource of changes	Degree of freedom	Mean squares ** F
Treatment	3	91 13415.77
Error	8	16.227

Significant at 1%, CV=6.97

According to Table 3, at least two groups of these 4 treatments are statistically different at the level of 1%.To determine which one of these treatments has a significant difference with other ones, the averages were compared by Duncan method.

Table 4. Comparison of the mean treatments (vitamin B12 , vitamin C , distilled water and *crataegus oxyacantha* extract (65% ethanol and 35% water *in vitro*)

Treatment	**The average means of rotation**	**rank**
Distilled water rotation	306.67	A
Vitamin B12 rotation	286.67	B
Vitamin C rotation	230.00	C
crataegus oxyacantha extract(flavonoids)	275.00	D

According to the Table above, by existence of distilled water as a control, plant bionanosensor able to detect vitamin B12, vitamin C and *crataegus oxyacantha* extract (flavonoids) Antioxidants from each other is likely to be 99%.

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Distilled water showed the highest degree of rotation, because distilled water absorption is higher than other treatments and and vitamin C showed a lower degree.

Based on the SEM method, the plant sample used in bio nano sensor 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.

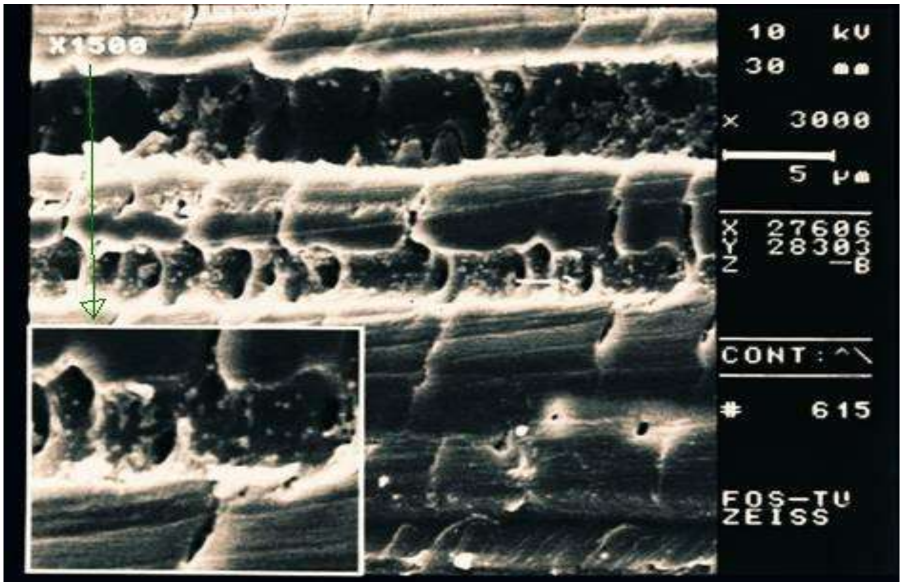

Fig3.Longitudinal-section, the image of electronmicroscope of plant sensors based on SEM method (Moradi ,f ,et al,. 2018, Moradi,f,..2019).

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

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 transportable in any conditions.
4. Unlike other methods, it did not use any electrical energy source, because Treatments 02- 0.025 mg flavonoid on hyperosoide, 1 mg vitamin C, 5 mg vitamin B12 and 0 mg flavonoid (distilled water as a control) act as a biosensor energy source.
5. If get optimized, it would be possible to detect all the antioxidants.
6. It is high speed chromatography than HPLC, chromatography and spectroscopy methods.

Suggestions:

If a biosensor would made based on 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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