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Evaluation of the Antioxidant Activity of Rosa canina Plant Fruit

Abstract

Three types of Rosa canina plant extracts were prepared. The antioxidant activity of the first prepared solution was measured using a UV spectrophotometer in the bioengineering laboratory. The resulting graph shows that the DPPH free radical is blocked by the antiradical antioxidant contained in the rosehip plant extract. We then analyzed the resulting graph using Origin Corporation software and obtained a new graph. Origin Corporation software offers a wide range of features, making it easy to analyze the laboratory results. The software analyzes the results by performing mathematical calculations and provides more detailed information. These analyses allow for the evaluation of the potential antioxidant properties of the Rosa canina plant and enhance the understanding of its potential use in medical fields. The findings could contribute to the future application of rosehip in therapeutic practices and health-related research.

Keywords: *Rosa, plant extract preparation, antioxidant activity, UV spectrophotometry, Antiradical antioxidant*

Introduction

In recent years, the health benefits of plants, particularly their antioxidant activities, have been extensively studied. Rosa canina (dog rose) is known for its rich content of vitamins, minerals, and bioactive compounds. The extracts of this plant are especially significant for their antiradical properties, which can play a key role in combating free radicals. Free radicals are responsible for a range of diseases, including cancer, cardiovascular diseases, and aging. Therefore, the study of antioxidant compounds derived from plants is crucial for health preservation and disease prevention. In this study, the antioxidant activity of extracts from Rosa canina fruits was evaluated using various methods. Two different types of extracts were prepared, and their antioxidant properties were measured using a UV spectrophotometry device. The results demonstrated that the dog rose extract effectively blocks DPPH free radicals. Additionally, the analysis of the resulting graphs was performed using Origin Corporation software, which facilitated the interpretation of the data through mathematical calculations. This tool allows for more efficient and precise analysis of laboratory results.

The aim of this study is to measure the antioxidant potential of Rosa canina extracts and identify how this activity changes across different preparation stages. The findings could further highlight the health benefits of this plant and contribute to its wider application in the medical field.

The growing interest in natural antioxidants has led to an increasing number of studies focusing on plant-based compounds as potential alternatives to synthetic antioxidants. Rosa canina, with its rich biochemical composition, presents itself as a valuable source of natural antioxidants. Its fruit is known for high levels of vitamin C, flavonoids, and phenolic compounds, all of which are potent antioxidants that can neutralize harmful free radicals in the body (Arshad, 2017).

In this study, we aimed to explore the effectiveness of Rosa canina fruit extract in scavenging free radicals, particularly focusing on its ability to inhibit DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals. DPPH is a commonly used compound in antioxidant assays due to its stability and ability to measure the scavenging activity of antioxidants. The UV spectrophotometric analysis provided

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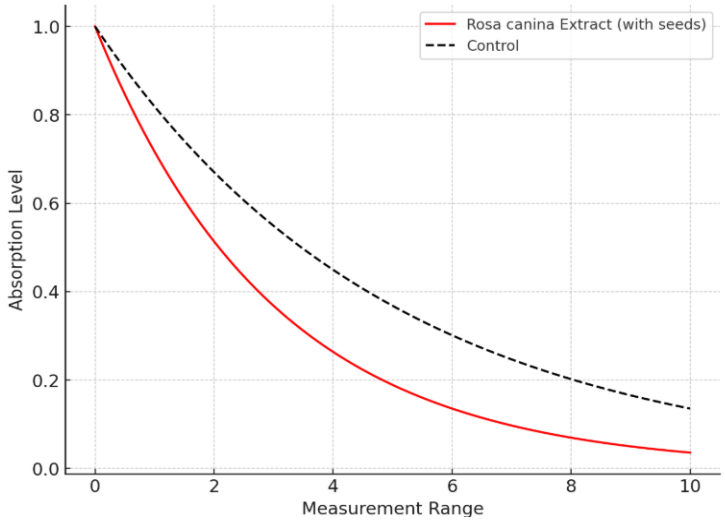
insight into the degree of radical inhibition by the extract, while the use of Origin Corporation software allowed for precise graphical representation and interpretation of the results (Shahidi, 2014).

Additionally, the preparation methods for the Rosa canina extracts were carefully controlled to ensure minimal degradation of bioactive compounds. Extracts were prepared both with and without seeds, and the influence of different preparation techniques, such as the use of fresh fruits and leaves, was investigated. The results of this study could provide further evidence of the potential health benefits of Rosa canina, particularly its antioxidant properties, which may offer therapeutic benefits in preventing oxidative stress-related diseases.

By analyzing the antioxidant activity of Rosa canina through UV spectrophotometry and advanced data processing tools, this research contributes to the growing body of knowledge surrounding the health-promoting properties of plant-based antioxidants and supports the potential use of Rosa canina in various therapeutic applications (Lankin, 2001).

Figure 1

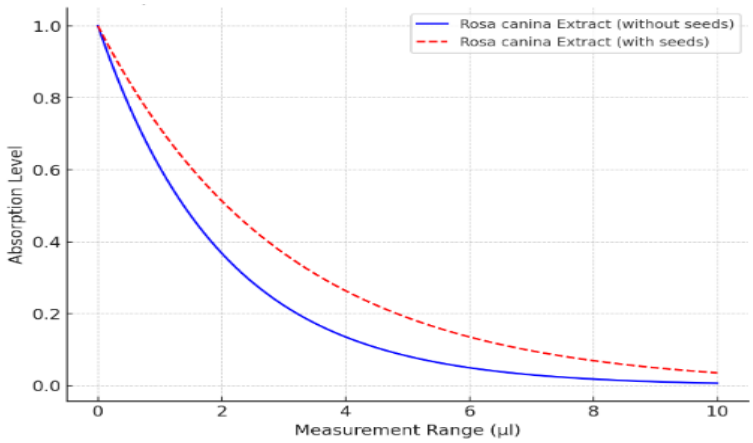
Evaluation of Rosa canina fruit extract with seeds



First, we evaluated the antioxidant activity of rosehips extract with seeds. The red line in the graph presents absorption level of our whole extract, the black thin line is control line for the comparison. From this graphic it is clear to us how high is antioxidant activity of dog rose.

Figure 2

Comparison of extract Rosa canina with and without seeds



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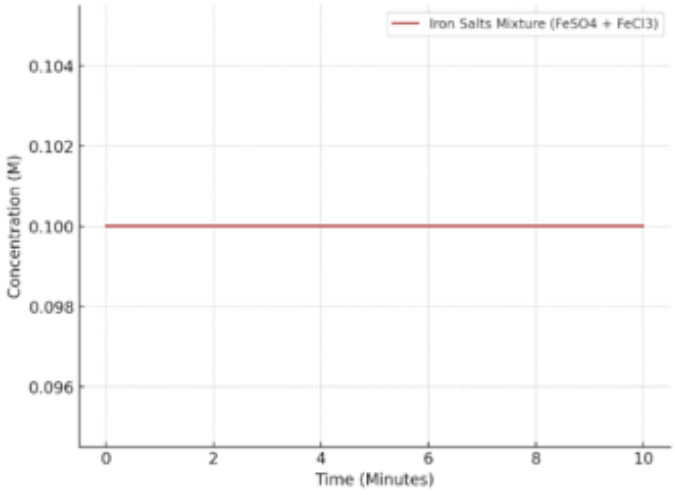
We then tested the seedless rosehip extract and found that it had higher antioxidant activity than the rosehip extract with seeds, as shown in the graph above. The reason for this is the presence of cyanide in the seeds. Obviously, some species of Rosa contain cyanide, but the amount of this is not very high. So, if it will be use in high concentration it can be dangerous ,because this composition is poisonous. Cyanide can decrease the level of antiradical activity of plant extract. Both of these extracts pass through the line of control, it means inhibition proccess higher than 50 %. Blue line's absorption happens in 1µl,the whole extract's inhibition happens in 3.5 µl.

Research

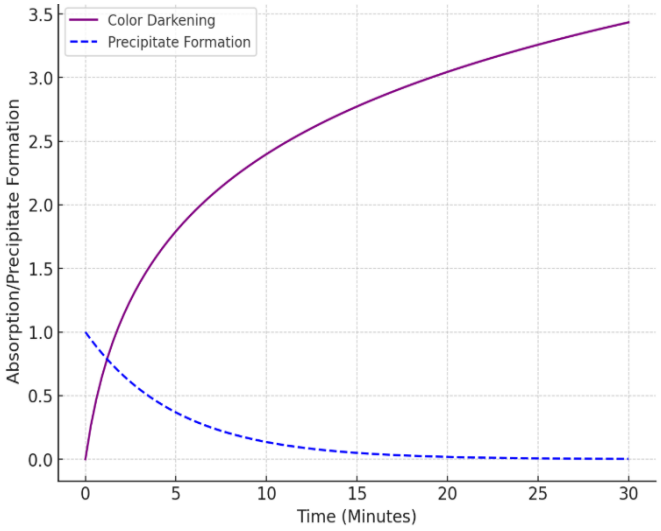
To carry out green synthesis of iron oxide nanoparticles, we first prepared iron oxide salts. Each of them is mentioned in the second section. We first used a mixture of both salts, both $FeSO_4$ and $FeCl_3$, in the synthesis of iron oxide nanoparticles. We took the concentration of each of them as 10^{-1} M.

Figure 3
Iron salts mixture



As shown in the figure, we mixed the iron oxide salts and poured them into a glass flask, then placed them on a magnetic stirrer and simultaneously heated to 70 degrees for half an hour. Then a drop of rosehip extract was added.

Figure 4
Adding process of plant extract



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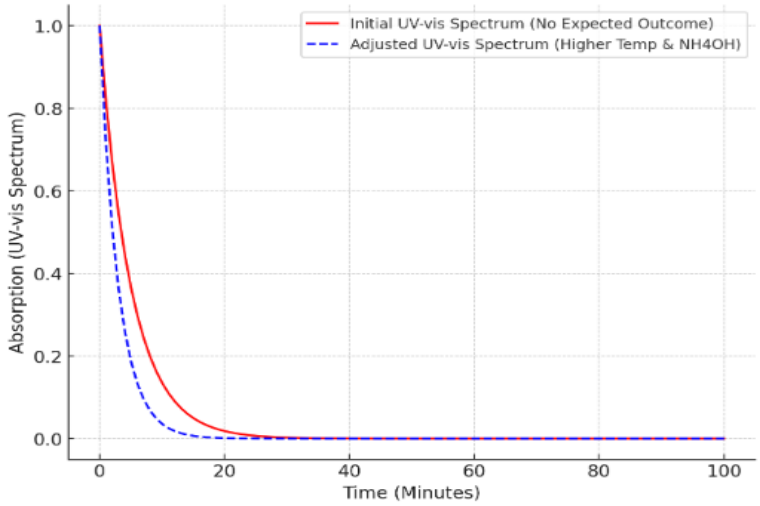
As the solution was added, the colour of the solution began to darken. We switched off the heater and continued the process for another 15 minutes, eventually the entire solution darkened. At this time a precipitate was formed.

Figure 5
UV-vis spectrum of IONPs

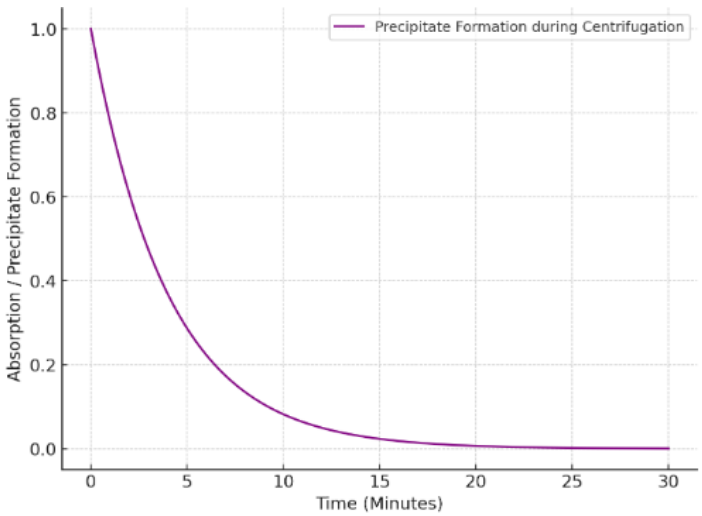


Our first result did not meet our expectations. The second time we changed the concentration and ratio to 2:1. The changes we made this time were to raise the temperature to 80 degrees and use NH_4OH as an additional stabilizer.We put to the glass 10 ml of 0.25 M $FeSO_4x7H_2O$, 20 ml of 0.24 M $FeCl_3x6H_2O$,then was added 50 ml of extract under continuous mixing for 30 minutes. Then 30M(12 M) of NH_4OH was added to the mixture continued for 1 hour at 80 C until the color of the solution changes to dark (Arshad, 2017).

After the changes were made in the second attempt, the reaction proceeded with improved results. The temperature increase to 80°C and the addition of NH4OH as a stabilizer played a crucial role in enhancing the synthesis of iron oxide nanoparticles (IONPs). As the mixture continued to stir for one hour, the color gradually transitioned from a light yellow to a darker hue, indicating the successful formation of nanoparticles (Karami, 2015).

The darkening of the solution is a visual indication of the nanoparticle formation, as the Fe(III) ions in the solution reduced and began to form iron oxide structures. The addition of NH4OH likely provided the necessary alkalinity to facilitate the precipitation of iron hydroxide, which then further transformed into iron oxide nanoparticles under the heated conditions.

Figure 6
Final solution



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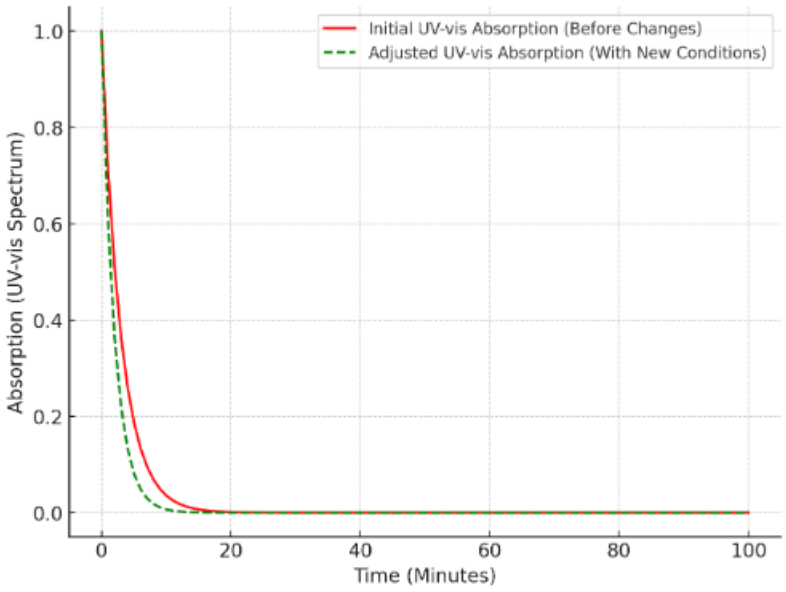
The formation of a precipitate is observed. We centrifuged these samples with 6000 rpm for 20 minutes.

Figure 7
UV-vis absorbtion of IONPs



Once again as a result of in-depth research we started to synthesise iron oxide nanoparticles. This time we changed the environment, temperature, pH and reagents for work.

First of all, we prepared extract from freshly harvested rosehip fruits from Astara district. This time we purified the extract from seeds and added it to the solution of green rosehip leaves. As mentioned above, we added 250 ml of distilled water to 4 grams of rosehip fruit and 4 grams of rosehip leaves. Waited 1 week to get the extract, in this case it is more natural and the ingredients can be obtained without decomposition. Many biochemical components are known to break down at temperatures over 45 degrees centigrade.

After preparing the extract from the freshly harvested rosehip fruits and leaves, we ensured that the extraction process was conducted at room temperature to preserve the natural bioactive compounds. The extraction was left for a week to allow sufficient time for the components of the rosehip fruits and leaves to fully dissolve into the distilled water. This process was carefully controlled to prevent any degradation of sensitive compounds, as many biochemical substances, such as vitamins and flavonoids, are known to decompose when exposed to temperatures above 45°C (Shahidi, 2014).

During the extraction period, the mixture was kept in a dark, cool place to protect it from light and heat, which could also cause the breakdown of certain compounds. After one week, the resulting extract was filtered to remove any solid particles, leaving behind a clear solution rich in the active ingredients from the rosehip fruits and leaves. This extract was then ready to be used for further synthesis processes or analyses.

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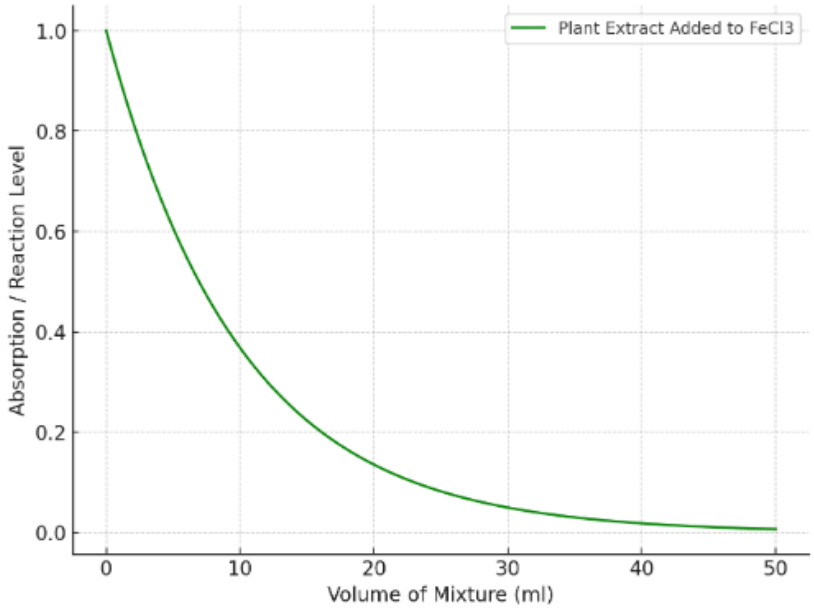
Figure 8
Rosa canina plant extract



In the second step we prepared a solution of iron oxide salt. Our solution is prepared from 0.1M $FeCl_3$ salt. To 50 ml of 0.1M $FeCl_3$ we add 50 ml of our plant extract drop by drop. The resulting mixture is placed on a magnetic stirrer. The mixed reagents are taken at room temperature in the ratio 1-1.

Dependence of iron oxide nanoparticles synthesis on pH, concentration and temperature

Green synthesis of iron oxide nanoparticles is more complicated than that of silver and gold nanoparticles. The reason for the difficulty is that iron is an active metal. As mentioned earlier, the absorption of iron oxide nanoparticles depends on the environment. First of all, the synthesis solution can change the direction of the reaction depending on the pH level of the environment. The synthesis of nanoparticles occurs through oxidative reduction reaction. For green synthesis to occur, the environment must be alkaline. We observed that when silver nanoparticles were synthesised, they were obtained in an acidic environment. However, it is believed that in the synthesis of iron oxide nanoparticles, on the contrary, a low pH value reverses the direction of the reaction (Karami, 2015).

In general, since iron is a metal of variable valence and active, its green synthesis is slower than that of passive metals. It can be obtained faster in chemical synthesis because the reagents can be selected. It becomes difficult to control during green synthesis because the reactions occur depending on the composition of the plant. In our research studies, we obtained green synthesis of iron oxide by changing the pH of the medium. The percentage of NaOH solution was 11. If we look at other studies, we see that in the synthesis of iron and iron oxide, hydroxides such as NaOH and NH_4OH are used to create an alkaline environment. The second influencing factor was temperature. According to many years of research, it is known that if a plant extract is boiled above 40 degrees, the composition can change dramatically, leading to the dissolution of substances. In our research, we boiled the extract prepared from rosehip fruit to 70 degrees. However, we received positive feedback by taking the extract using ancient methods, waiting a few days without boiling it. When synthesising iron oxide we did not boil it to 80 degrees, we got the best results at 45 degrees because our extract was not damaged at this time.

The third influencing factor was concentration. We prepared saline solutions with 10^{-2} M and made them in a 2-1 ratio, then mixed them together, but this did not help. Then we prepared 0.25 M and mixed again, the result again did not meet our expectations. Finally, by varying both the concentration and the ratio using the same salt we got the result we wanted. At this time, we brought the concentration to 0.1M using only $FeCl_3x6H_20$ salt. Then when we mixed it with the

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extract, we took it in 1-1 ratio and our result showed positive response as above. In conclusion, each factor needs to be managed separately, otherwise the result may not live up to expectations.

Analysis of Results

Based on the above mentioned literature, it can be said that nanomaterials produced by green synthesis method related to biological synthesis of nanoparticles are more environmentally friendly, cheaper, accessible and less toxic. Iron oxide nanoparticles produced by biological synthesis have a wide range of applications.

These are mainly in medical and industrial fields. Iron oxide nanoparticles, which play an important role in nanoencapsulation of medical drugs, are now very topical for the delivery of targeted nanomedicine. According to our results, iron oxide nanoparticles depend on a number of factors.

First of all, the method of preparation of plant extract plays an influential role in the resulting medium. Based on our observations, we can say that the extract prepared by us from the fruits of freshly harvested Rosa canina plant was more convenient. The absence of a heater during the preparation of the extract had a positive effect on the synthesis (Karami, 2015).

The synthesis process can be accelerated by mixing the prepared solutions of the two iron salts or by adding them to the extract separately. As is known, the rosehip plant contains various and many acids. One such acid is vitamin C or ascorbic acid. Due to its large amount, it creates an acidic environment in the extract.

Iron oxide nanoparticles are not synthesised in an acidic but in an alkaline environment. Hence we got some results that did not fulfil our expectations. Then we added NaOH to the medium, brought the pH to 11 and successfully completed the synthesis process. We took 0.1 M of FeCl3x6h2O salt solution. In our previous studies, 0.25 M and 10-2 M were taken. However, 0.1 M synthesised better. Nanoparticles are synthesised at temperature. Iron oxide nanoparticles are better synthesised at 45 degrees. The production of nanoparticles was confirmed by UV-Vis spectrophotometry. It has been known for many years that the rosehip plant is a strong antioxidant. For this reason it is widely used in medicine and pharmacology.The extract was obtained by purifying the seeds of the rosehip plant and then it was evaluated in UV-vi (Arshad, 2017).

Both with and without seeds were observed for comparison. Rosehip extract without seeds showed higher antiradical properties. The reason for this was the cyanide contained in the seeds.

Conclusion

1. Iron oxide nanoparticles were synthesised from Rosa canina plant fruits collected in Astara district. Firstly, plant extract was prepared and we did two samples of extract: extract with seeds and without seeds. It was done for comparison.
2. The influence factors in synthesis were tested alternately. As we know in synthesis process of nanoparticles influence factors play crucial role, because it can change the direction of synthesis process.
3. During synthesis process more intensive IONPs synthesis was observed at 45 degrees. It is well known that in green synthesis process nanoparticles are forming in higher temperature very well.
4. By using pH-meter we measured the pH of mixture. Iron oxide nanoparticles was synthesised at pH 11. For comparison we did same measurement with silver nanoparticles solution, we got result which shows silver nanoparticles formation could be in lower pH. But, iron oxide nanoparticles only in pH 11 can synthesizes.
5. The antioxidant capacity of the extract prepared from rosehip fruit was measured by UV-vis spectrophotometry. Rosa canina plant has high antioxidant activity that’s why we choose this plant for study object.The synthesized nanoparticles solution also was measured, but unfortunately UV-vis spectrophotometry couldn’t evaluate the antioxidant activity.
6. The seedless rosehip fruit extract was found to have higher antioxidant capacity. It happens because, seeds of Rosa canina have a few toxic biochemical compound which decreases antioxidant activity.

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7. Iron oxide nanoparticles were rapidly synthesised in 0.1M solution. It was observed that, in other concentrations iron oxide nanoparticles didn't synthesis.

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