TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Effect of harvesting time of Salvia Absconditiflora on the antioxidant activity of it.

BIOLOGY EXTENDED ESSAY

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ABSTRACT:

The aim of the experiment was to investigate if there is a relation between the antioxidant activity of the *S. Absconditiflora* and the harvesting months of *S. Absconditiflora* (April, May, June and November) by looking the radical scavenging activity with the DPPH method.

My research question was: "How does the antioxidant activity of *Salvia Absconditiflora* that is cultivated in different months (April, May, June and November) change as indicated by the DPPH method in which the free radical scavenging activity is detected?"

It was hypothesized that the antioxidant activity of *S. Absconditiflora* changes as it is harvested in different months (April, May, June and November). It is expected that the antioxidant activity in April and May will be the strongest as they are the spring months and the antioxidant activity in November will be the lowest as it is the fall month.

In order to test the hypothesis and to answer the research question, the DPPH method used to determine the DPPH radical scavenging activity of *S. Absconditiflora* solutions. The salvia extract, DPPH solution and *S. Absconditiflora* extracts' solutions for the DPPH measurements were prepared. Radical scavenging activity were found for all solutions of both *S. Absconditiflora* and quercetin. The results were found by using the formula taking quercetin as a standard & control and percentage radical scavenging activity was calculated.

According to data, the percentage radical scavenging activities of *S. Absconditiflora* cultivated in different months were compared. As the vitamin and carotene amounts differ due to flowering, the antioxidant activity showed difference. *S. Absconditiflora* cultivated in November showed the least antioxidant effect as the mean value is 84.71, followed by *S. Absconditiflora* in April as the mean value is 87.88 . *S. Absconditiflora* in June and *S. Absconditiflora* in May showed the strongest antioxidant activities. June has the mean value 86.65 and May has the mean value 84.30. The p value is smaller than 0.05 and if the p value from anova is smaller than 0.05 so it is found that there is a significant mean difference between the antioxidant activity and harvesting time. With the help of experiment it is found that *S. Absconditiflora* is affected by the harvesting months. This study shows the importance of harvesting time on the antioxidant activity of *S. Absconditiflora.*

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INTRODUCTION:

The first time I was confronted with the topic was when my teacher gave me sage tea when I was flu. She said it is a perfect cure for healing. I wondered about what are the sage's benefits and searched about it. Mostly the articles were focusing on the free radical scavenging capacity of sage. I was excited because I learned in biology class that, antioxidant activity is very beneficial for the health of human beings. Therefore, I have decided to search the antioxidant activity of *Salvia* plant.

Salvia is the largest genus of plants in the mint family. *Salvia* parts have many notable plant-derived chemical compounds, essential oils, minerals, vitamins that are known to have disease preventing and health promoting properties. [1]

In the first century CE Greek physician Dioscorides reported that the aqueous decoction of sage stopped bleeding of wounds and cleaned ulcers and sores. He also recommended sage juice in warm water for hoarseness and cough. Internally, a tea made from sage leaves has had a long history of use to treat sore throats and coughs; often by gargling. It was particularly noted for strengthening the nervous system, improving memory, and sharpening the senses. It is considered a useful medicine in typhoid fever and beneficial in biliousness and liver complaints, kidney troubles, haemorrhage from the lungs or stomach, for colds in the head as well as sore throat, quinsy, measles, for pains in the joints, lethargy and palsy. [2]

A study in Edremit Gulf of Balıkesir *Salvia Tomentosa* leaves are prepared by infusion and drunk two times a day in the treatment of cold, flu and tonsillitis. Besides these benefits for health, there are laboratory studies that show antimicrobial, anti-inflammatory, antioxidant and many other properties of *Salvia* genus.

Salvia contains very good amounts of vitamin A and it is a powerful natural antioxidant and is essential for eye-sight. Fresh sage leaves are a good source of antioxidant vitamin, vitamin C. [3]

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[1] *The Gardener's Guide to Growing Salvias*. Workman Publishing Company. p. 17*The New Book of Salvias*. Timber Press. p. 18.

[2] http://www.herbwisdom.com/herb-sage.html

[3] http://www.nutrition-and-you.com/sage-herb.html

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. They can cause cancer, heart disease, decline in brain function or decline in immune system. To prevent free radical damage the body has a defense system of antioxidants. [4]

An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions.[5]

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (as it traps the free radicals). Plant sourced food antioxidants like vitamin C, vitamin E, carotenes have been recognized as having the potential to reduce disease risk. [6]

There are several different methods for measuring the antioxidant activity (see appendix 8). However DPPH method will be used in the experiment.

DPPH method is highly used for detection of radical scavenging capacity of foods such as fruits, vegetables, juices and wines. It is a reproducible technique and needs only UV-spectrophotometer to do measurements. Due to its free electron delocalization, DPPH is a stable nitrogen free radical. This delocalization gives it a deep violet color in solution which can be measured with UV-spectrophotometer in range of 515-530 nm wavelengths. [7]

When DPPH solution is mixed with a H+ donor, the molecule turns in to non radical reduced form. The color of solution converts into a pale yellow color due to its picryl group still present. The basis of the method depends on the measurement of this decolorization. The reaction in this process is biphasic. That starts with a fast decline in absorption in the first few minutes, continues with a slower reaction and results with equilibrium.

$$DPPH_{+} + AH \longrightarrow DPPH\text{-}H + A_{+}$$

$$DPPH_{+} + R_{+} \longrightarrow DPPH\text{-}R$$

[4] http://www.rice.edu/~jenky/sports/antiox.html

[5] . "Oxidative stress: Oxidants and antioxidants". *Experimental physiology* **82** (2): 291–5.PMID 9129943.

[6] http://www.medlabs.com/Downloads/Antiox_acti_.pdf

[7] chen et al., 2013; Molyneux, 2004; Villano et al., 2007

As illustrated above, $DPPH_+$ is free radical AH is antioxidants in foods, A_+ is newly formed radical species, and DPPH-R is final products.(brand Williams et al., 1995)

The antioxidant activity can be affected by the cultivating, ripening factors as the vitamin, carotene amount can differ. During my research I investigated whether the antioxidant activity of Salvia affected by the change in harvesting months. As I researched about it I found that tomatoes also affected by the ripening factor. When they get red the lycopene amount increases.[8] Because of these reasons I decided to look for the antioxidant activity of Salvia plant that is harvested in different months.

Many studies were done about antioxidant effects of this plant but I want to look at the antioxidant activity of *Salvia* that is grown in different months. The salvia is cultivated during spring and fall. Therefore, I have chosen this topic for my extended essay. My research question is "**How does the antioxidant activity of *S. Absconditiflora* that is cultivated in different months (April, May, June and November) change as indicated by the DPPH method in which the free radical scavenging activity is detected.**

[8] http://www.motistel.com.tr

HYPOTHESIS:

Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. *Salvia* plant has many beneficial effects for health and beside all these, it has also antioxidant activity that decreases the activity of free radicals.[9]

Salvia plant is grown in different season. The abiotic or biotic factors can show difference in different months like water, minerals nutrients according to rate of photosynthesis, light,… These factors can affect the vitamin c, vitamin e or carotene contents . Also the difference in flowering causes the change in the contents of the substances. As there can be difference between these factors season by season the antioxidant activity can be different. [10]

It was hypothesized that the antioxidant activity of *S. Absconditiflora* changes as it is cultivated in different months (April, May, June and November). It is expected that the antioxidant activity in April and May will be the strongest as they are the spring months and the antioxidant activity in November will be the lowest as it is the fall month. The conditions like temperature, sunlight, water amount (the rain amount), etc. factors are optimum in spring so the development of the plant will be the best and the vitamin, carotene amount will be higher. However in autumn the conditions will be less optimum and as the plant patch off the leave amount and the photosynthesis amount decreases.

[9] http://www.nutrition-and-you.com/sage-herb.html
[10] http://www.nutrition-and-you.com/sage-herb.html

METHOD DEVELOPMENT AND PLANNING:

Before doing my extended essay experiment I researched for a proper topic and found that *Salvia* has antioxidant activity which is good for human health. I decided to look for the change in antioxidant activity of *Salvia* plant that is harvested in different months.

Salvia plant has lots of benefits and used to heal several things. There are several species of *Salvia* found in Turkey. *S. Absconditiflora* is one of the endemic *Salvia* species grown in Turkey which is consumed as herbal tea. Because of the presence of high amounts of vesicle on their leaves, *S. Absconditiflora* is very rich in active compounds. Also it is found in highly amounts in Middle East Technical University campus forests located in Ankara. So the species will be used in experiment is *S. Absconditiflora.*

S. Absconditiflora leaves were collected in different months since the experiment is about the antioxidant effect of the plant grow in different months. The plant is collected in April, May, June and November. Spring is the best season for plant growth so we have collected *S. Absconditiflora* mainly in April and May. Plant has been collected also in the beginning of the summer and in the beginning of the fall in order to see if the antioxidant capacity of the plant is still active. *S. Absconditiflora* leaves were collected from forested land near Biological sciences Department in METU campus in April, May, June and November. 25m2 quadrats were used to make sure that the *Salvia* will be collected from exactly the same location. The lattitude, distance to road, the time of the day, etc. were kept the same.

Before doing the experiment to find out " How does the antioxidant activity of *Salvia Absconditiflora* that is cultivated in different months (April, May, June and November) affected as indicated by the DPPH method in which the free radical scavenging activity is detected." I decided where will be the experiment done. I researched the proper laboratory which allows me to perform the necessary methods. I decided to do this experiment in Prof. Dr. Tülin Güray's laboratory in Middle East Technical University, because the conditions are good and all the materials and machines that I need in my experiment available in there as it is a developed laboratory. The variables like light, temperature, pressure, etc will stay constant.

Another problem was how to make an extract from the *Salvia* plant. After researching for the most appropriate way I decided make an extract by putting the plant into the water extraction machine for 2 days. This method is a less priceless and simple method to make an extract. Also as Salvia is a plant which is drained as a tea and can be drank, this method was done with water. After making the extracts they were put into the freezer(-80°C) in order to prevent deterioration of the extracts.

Another problem was to find the proper method for the experiment. There are several methods for looking the antioxidant activity. DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable and need not be generated. As I'm not an experienced scientist I have to choose the most easy and reproducible for my extended essay.

In various laboratories different protocols are applied which differ in initial DPPH concentration (22.5μM-250 μM), incubation time (5- 60 minute) and reaction solvent (ethanol, methanol).

It is very necessary to find the optimum incubation time for solution in which reaction should be followed until reduction of DPPH has reached to plateau. For this reason I have tested several incubation times like 10 min 20 min 30 min 40 min before constructing a valuable protocol. After several trials, incubation time was selected as 40 min as DPPH has reached to plateau in that time. When we concern solvent types, it was found that both ethanol and methanol are good solvents for the assay, but as methanol is more toxic and has more risks during the experiment ethanol used in the experiment.

Since *S. Absconditiflora* leaves were collected in different months, DPPH radical scavenging activity was measured for each month's extracts. For each month's different leaf extract concentrations 0.25, 0.5, 0.75, 1,2 and 4 ml/ml were used at the beginning to see if the % RSA is increasing properly as the concentration increases. I used the concentration in which the data are the most accurate and it is 4 mg/ml for my trials.

Besides looking DPPH scavenging activity of *S. Absconditiflora* in 4 months, I also looked at the quercetin's (the control of the experiment) antioxidant activity as a control. Quercetin has displayed the ability to prevent the oxidation of low- density lipoproteins by scavenging free radicals and chelating transition metal ions. But the antioxidant activity of quercetin is much more than the *Salvia*. So the quercetin helps to compare the *S. Absconditiflora*'s antioxidant activity and check whether the values are correct or not. [11]

There are some difficulties that I have faced during the experiment. Firstly, the concentration of DPPH that is needed is too small (0.0001gr) that it was hard to take the accurate measure. I used the Mettler Toledo balance which is very sensitive (±0.00001). Then I dissolved it in 99.5% ethanol with the help ultrasonicator for 30 to 40 minutes since it is hard to dissolve DPPH. At the same time the *S. Absconditiflora* extracts were dissolved in distilled water in different concentrations. Another problem was to make sure that the materials were dissolved homogenously. To eliminate the problem, the folc vortex machine was used before the trials to mix the samples homogenously. The last problem appeared when measuring the DPPH radical scavenging was that the instrument UV-spectrophotometer. The cuvette holder had a problem for accurate measurements. For this reason, the test tubes should be properly placed into the cuvette and checked.

[11] http://www.jyi.org/issue/a-review-of-quercetin-chemistry-antioxidant-properties-and-bioavailability/

Materials used in the experiment:

- Distilled water (dH2O)
- 0.0005 gr DPPH
- Quercetin (375 mcg/ml)
- *Salvia Absconditiflora* extract (5 gr)
- Water (200 ml)
- UV-spectrophotometer
- Ultrasonicator
- Mettle Toledo (very sensitive weight)
- Folc vortex
- Appendorf (pipette)
- Ethanol (%99.5)
- Test tubes
- Quadrat(25m2)

METHOD:

I. Preparation of *Salvia* extract: (see appendix 5)

II. Preparation of DPPH solution (see appendix 6)

III. Preparation of *S. Absconditiflora* extracts' solutions for the DPPH assay (See appendix 7)

IV. Preparation of quercetin (control) solutions for DPPH activity measurement

V. Experiment is conducted

Absorbance of DPPH was measured against ethanol at 516 nm in order to check whether it is ready or not for the experiment. After that, 100 µl of each concentration was mixed with 1400 µl DPPH solution in the tube in duplicates, vortexed and they were incubated at room temperature in dark for 40 min, which was optimized before. Tubes were poured into cuvette and measured at 516 nm with spectrophotometer (Shimadzu UV-160A, Japan) against 99.5% ethanol as reference cuvette. Sample blank absorbance was measured in order to eliminate the absorbance effects of *S. Absconditiflora* itself. Quercetin was also measured in different concentrations as a positive control.

The result of the assay is interpreted using the EC_{50} concentration parameter, which is the concentration of the *S. Absconditiflora* extract that causes 50% loss of DPPH free radical activity. It is calculated from % RSA (radical scavenging activity percentage) versus concentration curve. (see appendix 1)

- The steps are done for April, May, June and November in *S. Absconditiflora* and for quercetin as a control. For every month and quercetin 3 independent trials were done. (5×3=15 trials)

DATA COLLECTING AND PROCESSING:

APRIL	4.00	0.0001	0	1.516	0.232	0.227	0.236
MAY	4.00	0.0001	0	1.516	0.184	0.178	0.189
JUNE	4.00	0.0001	0	1.516	0.202	0.200	0.205
NOVEMBER	4.00	0.0001	0	1.516	0.238	0.234	0.241

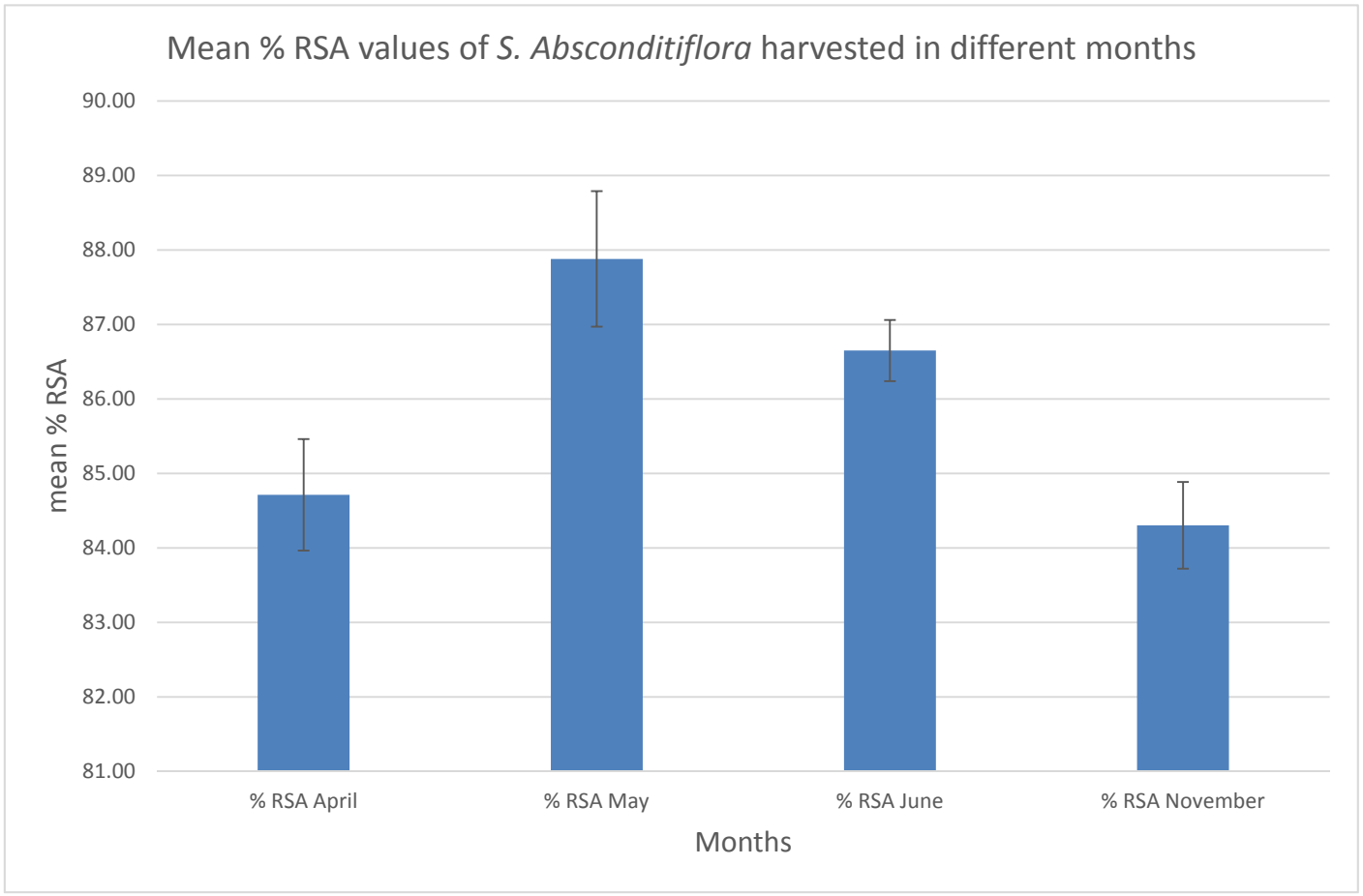
Table 1: Table shows the concentrationof *S. Absconditiflora*, amount of DPPH, sample blank, absorbance blank and absorbance sample values in 516 nm of antioxidant activity of *S. Absconditiflora* that were harvested in different months.

	% RSA April	% RSA May	% RSA June	% RSA November
TRIAL 1	84.68	87.85	86.66	84.25
TRIAL 2	85.03	88.26	86.81	84.56
TRIAL 3	84.43	87.53	86.48	84.10
MEAN	84.71	87.88	86.65	84.30

.

Table 2: Table shows the percentage radical scavenging activity of of *S. Absconditiflora* that are harvested in different months in 3 independent trials.

Graph 1:the mean values of percentage radical scavenging activities (%RSA) of *S. Absconditiflora* measured by DPPH method that harvested in different months.



DATA ANALYSIS:

MEAN:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

Where

n is the number of trials(in this experiment 3 for all months and concentrations.

X is the percentage scavenging activity of *S. Absconditiflora.*

STANDARD DEVIATION:

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \overline{x})^2}$$

Where:

n is the number of trials(in this experiment 3 for all months and concentrations)

Xi is the percentage radical scavenging activity

X is the mean value for the corresponding group

STANDARD ERROR:

$$\sigma_{\overline{x}} = \frac{\sigma}{\sqrt{n}}$$

Where:

σ is the standard deviation for all months.

X is the mean value for the % RSA of *S. Absconditiflora* in different months

	% RSA April	*% RSA May*	*% RSA June*	*% RSA November*
Mean	84,71	87,88	86,65	84,30
Standard Error	0,17	0,21	0,10	0,14
Standard Deviation	0,30	0,37	0,17	0,23
Count	3	3	3	3
Confidence Level(95,0%)	0,75	0,91	0,41	0,58

Table 3: The table shows the mean values, Standard deviations, Standard errors and the confidence levels of the % RSA values for the *S. Absconditiflora* that are harvested in different months.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	25,31913333	3	8,439711111	109,9397887	**7,67E-07**	4,066181
Within Groups	0,614133333	8	0,076766667			
Total	25,93326667	11				

Table 4: The table shows the single factor Analysis of Variance (ANOVA) statistical calculation.

EVALUATION:

The research question was "How does the antioxidant activity of *S. Absconditiflora* that is harvested in different months (April, May, June and November) change as indicated by the DPPH method in which the free radical scavenging activity is detected". It was hypothesized that the antioxidant activity changes as the *S. Abscoditiflora's* harvesting months change. It is expected that as the flowering varies and the vitamin c, carotene amounts differ the antioxidant activity shows difference.

S. Absconditiflora harvested in November showed the least antioxidant effect, followed by *S. Absconditiflora* in April. *S. Absconditiflora* in June and *S. Absconditiflora* in May showed the strongest antioxidant activities. The % RSA ranged between 84.10 and 84.56 with the mean value of 84.30 for *S. Absconditiflora* in November. The % RSA is ranged between 84.43 and 85.03 with the mean value 84.71 for *S. Absconditiflora* in April. The % RSA is ranged between 86.48 and 86.81 with the mean value 86.65 for *S. Absconditiflora* in June. Finally the %RSA is ranged between 87.53 and 88.26 with the mean value 87.88 for *S. Absconditiflora* in May.

My hypothesis was that the antioxidant activity shows difference as the harvesting months of *S. Absconditiflora* changes by looking the percentage radical scavenging activity with the DPPH assay. As shown in the table 4, p value which shows the single factor anova is 7,67E-07 which is smaller than 0.05. This value proves that my hypothesis was correct as it is smaller than 0.05 and there is a significant mean difference between the groups. Therefore the antioxidant value changes as the cultivating time for *S. Absconditiflora* changes.

The% RSA values were close to what I expected in the experiment. I have used the plants that were harvested in April, May, June and November. I expected that the antioxidant activity will be strongest in May and April and lowest in Nowember. As what I expected antioxidant activity of *S. Absconditiflora* in May is the strongest, however the antioxidant activity in June was higher that the antioxidant activity in April. Lastly the result of November was the lowest as I expected.

I also looked the antioxidant activity of quercetin by changing the concentrations of it as control. From looking the data (in appendix 2,3) we can say that the % RSA is increased as the concentration increased and also by looking the graph, it can be seen that the concentration is directly proportional with the antioxidant activity. So this proves that my experimental method to measure the antioxidant activity was correct. Despite the amount of quercetin was very small compared to *S. Absconditiflora,* the % RSA was close to *S. Absconditiflora*.

The bar graph shows that there is a significant difference between the antioxidant activity and the cultivating months of *S. Absconditiflora.* The error bars were drawn by looking at the confidence level. The confidence level is 0.75 for April, 0.91 for May, 0.41 for June and 0.58 for November. As the confidence level shows difference we can say that there can be random errors in the experiment.

The standard deviation and standard error values also show difference between the groups. Standard error is 0.17 for April, 0.21 for May, 0.10 for June and 0.14 for November. Also the standard deviation is 0.30 for April, 0.37 for May, 0.17 for June and 0.23 for November. As we can see the standard error and standard deviation is the lowest is June and the highest in May. Therefore we can say that there can be random errors. However, as the standard error was smaller than 0.5 for all trials, the experiment was consistent.

During the experiment, there were no unexpected occurrences that may affected thde results of the experiment. However, while looking the statistical data, anova results abd writing the essay I realized some systematic errors that might affected the results. Some probable systematic errors in the method were listed and suggestions for improvement the investigation have been made:

1. For measuring the antioxidant activity, I used the DPPH method for this experiment however there are several other methods for measuring the antioxidant activity. Besides DPPH method has benefits, this method also has some downsides. This method is limited because DPPH radical interacts with other radicals and the time response curve to reach the steady state is not linear with different ratios of antioxidant/DPPH. DPPH can only be soluble in organic solvent and the interference of absorbance from the sample compounds could be a problem for the quantitative analysis The absorbance of DPPH in methanol, ethanol and acetone decreases under light.[12]
2. The species of *Salvia*. I use the *S. Absconditiflora* in the experiment. However there can be other species of *Salvia* that has more antioxidant activity. This species may give more significant difference between the months.
3. The number of months. We looked at the antioxidant activity for four months because *Salvia* grows in these months. However we could choose another species that is enable to grow in different months. Because if there are more months to look for antioxidant activity then the datas can be more comparable and we can both see more differences and discuss about it.
4. More concentrations can be tested for quercetin to get more accurate results and more proper graphic to see the relation better. Also very the same concentrations that are used for *S. Absconditiflora* could be used in order to see the real difference between them.
5. The *S. Absconditiflora* extract was made from the leaves of the plant. However the extract should be taken from all parts of the plant because the antioxidant activity may show difference as the vitamin amount can show difference. If the extracts were taken from both branches and leaves the results could be more accurate and the antioxidant activities of other parts were also tested.

[12] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3551182/

6. The incubation time for DPPH could be different. The optimum incubation time for DPPH should be the time in which the reaction should be followed until reduction of DPPH has reached to the plateau. However I could only tested in 10, 20, 30 and 40 min and I could have tested more than 4 different times. 40 minutes may not be the proper time for completing the reduction. So If more times were tested the optimum incubation time can be found and then more accurate data which are closer to the literature value will be found.
7. In the UV-spectrophotometer instrument the cuvette in which the absorbance sample values were put had detection problems. In order to take more proper measurements another instrument that has proper cuvette should be used.
8. Different methods for finding antioxidant activity could be used and the results could be compared.

CONCLUSION:

My research question: "How does the antioxidant activity of *S. Absconditiflora* that is cultivated in different months (April, May, June and November) change as indicated by the DPPH method in which the free radical scavenging activity is detected." is answered in the light of the results of my study. There is a significant difference between the percentage scavenging activity and the cultivating months of *S. Absconditiflora*. The antioxidant activity of *S. Absconditiflora* in May is the highest while it is the lowest in November as it was expected. Although the method can be modified for more accurate results, the study can be considered as successful.

The reason I was moved to do my extended essay on this subject was the natural healing property of plants was excited me. As the plants especially *Salvias* have antioxidant activity by removing the free radicals, they can reduce the risk for chronic diseases including cancer and heart disease. There are lots of plants that have antioxidant activity but I limited it into Salvia plant. There are lots of studies for looking the antioxidant activity of Salvia, but as I wanted to make a difference from others I wanted to look at the change in the antioxidant activity with the change in harvesting months. So I moved on the study form this topic. The results of the study showed that there is a significant difference between the antioxidant activities and the cultivating months of *S. Absconditiflora.* So with the results it can be proved that my hypothesis was correct.

The results of the experiment can be helpful for everyday life. Salvia can be considered as a plant for natural healing. In the past it was used for cold, flu, etc. It is also found that Salvia has antioxidant activity. Antioxidant activity means it removes the free radicals which starts chain reaction and causes some diseases. It is also said that it reduce the risk of cancer, heart problems and so on. People could consume these plants instead of medicines for healing. Medicines have both side effects and can cause tolerance if it is taken in high amounts. Consuming plants for healing is a natural process and has no bad effects. If people use this plants to prevent diseases as they have antioxidant activity. In the experiment it is also found that the antioxidant activity can change as the cultivating month of plant changes. So people should also beware the effect of months. The agriculture should be done considering this effects and people should harvest these plants in the time by looking in which the antioxidant activity is the strongest so that the natural healing process will be more effective.

APPENDICES:

APPENDIX 1:

The following formula was used to obtain the radical scavenging activity percentage:

$$\%RSA = ([\text{Absorbance}_{\text{Blank}} - (\text{Abs.}_{\text{Sample}} - \text{Abs}_{\text{Sample Blank}})]/\text{Abs.}_{\text{Blank}}) * 100$$

Where

%RSA	:	Radical Scavenging Activity Percentage
Absorbance Blank	:	Absorbance of DPPH (1400 uL) and dH_2O (100 uL)
Abs. Sample	:	Absorbance of DPPH (1400 uL) and Sample (100 uL)
Abs Sample Blank	:	Absorbance of Sample (100 uL) with dH_2O (1400 uL)

In order to calculate EC_{50} value, %RSA was plotted against the Concentration of the sample. (EC_{50} : 50% loss of DPPH free radical activity)

APPENDIX 2:

concentration of quercetin (mcg/ml) ±1mcg/ml	amount of dpph (gr) ±0.000001	sample blank dOD/min	absorbance blank dOD/min	absorbance sample dOD/min		
25	0.000025	0	1.516	1.241	1.208	1.232
50	0.000025	0.011	1.516	1.122	1.113	1.118
100	0.000025	0	1.516	0.590	0.556	0.567
200	0.000025	0	1.516	0.504	0.502	0.508

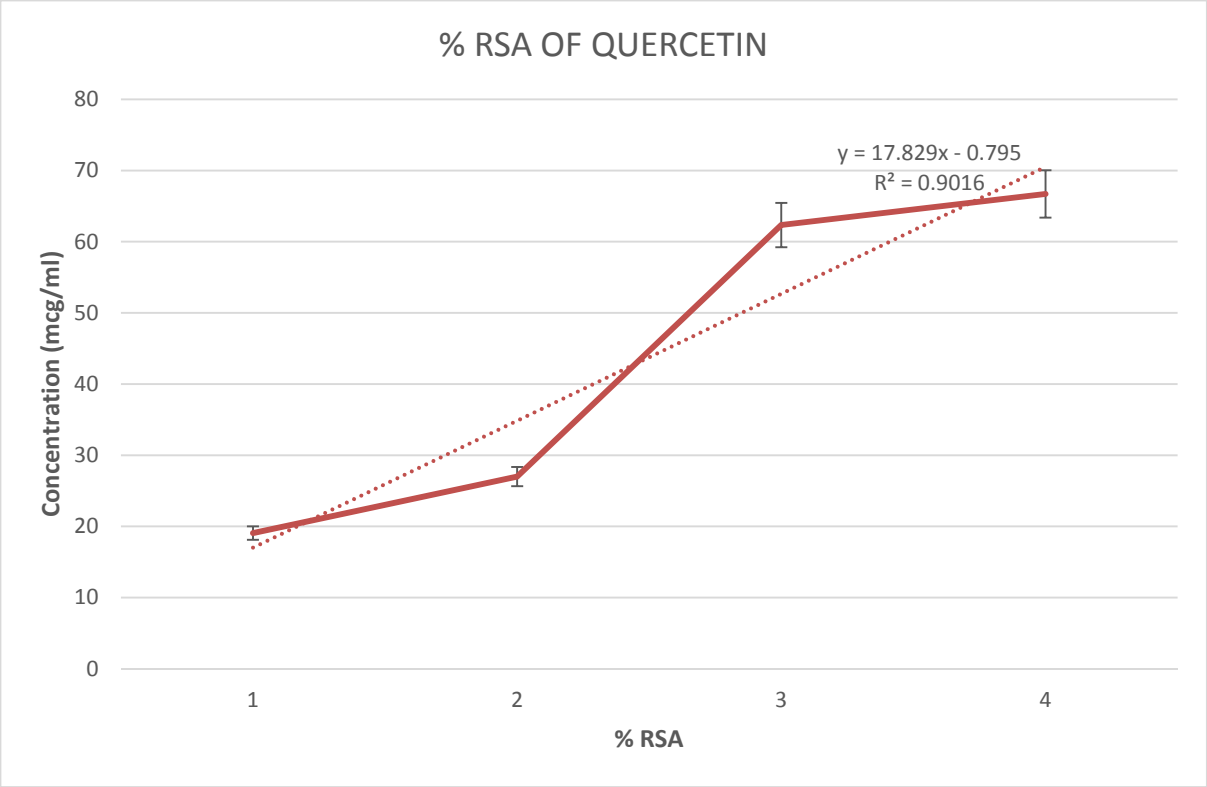
The table shows the amount of DPPH, sample blank, absorbance blank and absorbance sample for the different concentrations of quercetin.

APPENDIX 3:

Concentration(mg/ml) ±1 mg/ml	% RSA OF quercetin			Mean values
25	20.31	18.14	18.73	19.06
50	26.71	27.31	26.98	27
100	61.08	63.32	62.60	62.34
200	66.75	66.89	66.49	66.71

The table shows the percentage radical scavenging activity of quercetin in different concentrations and in 3 trials.

APPENDIX 4:



Graph of the mean percentage radical scavenging activity of quercetin in different concentrations.

APPENDIX 5:

Preparation of salvia extract:

5 gr of the *Salvia Absconditiflora* plant leaves were put into the water extract machine. The plant passed through the water vapor (20 ml) for 48 hours and obtained internal plant material. The water extract of *Salvia Absconditiflora* obtained.

APPENDIX 6:

Preparation of DPPH solution

For the experiment, 0.0001 g DPPH was dissolved in 99.5% ethanol in dark bottle. It was mixed thoroughly and waited in ultrasonicator for about 40 minutes.

APPENDIX 7:

Preparation of *Salvia Absconditiflora* extracts' solutions:

S Absconditiflora extracts were dissolved in distilled water. Then the serial dilutions at different concentrations were prepared. These are 0.25 mg/ml, 0.5 mg/ml, 0.75 mg/ml, 1 mg/ml, 2 mg/ml, 4 mg/ml. (less concentrations were used in quercetin since it was the control.)

APPENDIX 8:

Antioxidant capacity assay	Principle of the method	End-product determination
Spectrometry		
DPPH	Antioxidant reaction with an organic radical	Colorimetry
FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein[13]

[13] http://www.omicsonline.org/2161-1009/2161-1009-1-106.php

APPENDIX 9:

Picture 1: UV-spectrophotometer

Picture 2: Ultrasonicator

Picture 3: 3 different concentration of quercetin solutions for DPPH activity measurement

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