The effect of different ethanol concentrations on the cell membrane permeability in beetroots.

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Abstract:

This research will focus on the experiment of using different ethanol concentrations on beetroots in order to explore the effect of it on the membrane permeability. Ethanol (C2H5OH) is produced by the fermentation of carbohydrates which are in yeast cells, this is used as a solvent frequently. At 100% of ethanol concentration, it is a colorless liquid and is pure with a strong odour. A cell membrane's significant property is selective permeability, permitting some ions and molecules to pass freely through the membrane by excluding others which are not allowed to pass. The cell membrane is composed mainly of proteins and lipids in a structure which is a phospholipid bilayer. The structure of this allows flexibility to the membrane as it is in a fluid form, all of this is required for cell growth and movement. The cell membrane consists of integral proteins, peripheral proteins, glycolic proteins, cholesterol, channel and carrier proteins. Plant cells have vacuole, which in this case, the beetroot cell's vacuole contains a water-soluble red pigment which is betacyanin, giving the beetroot its red color. The cell membrane has tonoplast which surrounds the vacuole, tonoplast may be damaged causing the contents of the vacuole to spill. For the beetroot cell, when the tonoplast is damaged, the red pigment from the beets will leak out to the surroundings. The plasma membrane acts as protection and controls what substances can enter or exit the cell. Other than this, a colorimeter is a device used in the experiment to measure the absorbability of particular wavelengths of light by a specific solution. This concludes that as the concentration of ethanol increases, the absorbance of light would also increase.

Keywords - membrane permeability, ethanol concentrations, phospholipid bilayer, beetroot's cell membrane, plasma membrane

Research question:

What effect does the different concentrations of ethanol between a range of 0% to 99% with the same amount have on the beetroot to determine the permeability of it's cell membrane?

For this experiment, the independent variable will be the concentration of ethanol since we are trying to find the effects of different concentrations on beetroot in order to find the membrane permeability. Whereas our dependent variable is the size of beetroot and the amount of ethanol to make sure the experiment is reliable and that all solutions are the same. The Range for this investigation is the concentration of ethanol from 2% to 99%. The units used in this experiment are the arbitrary units.

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Personal interest:

The fact that ethanol is an alcohol, it is used in many areas for example in beverages like beer and wine as well as hand sanitizers. Ethanol was used most frequently in the year 2020 due to the pandemic of the coronavirus disease or COVID19. It is an infectious disease where people have been buying hand sanitizer to prevent spreading or getting the disease. Hand gels are used as a sanitizing agent to avoid the risk of infections and cross-contamination by microorganisms. I am very interested in experimenting with different concentrations of ethanol to see how it reacts with the plasma membrane and the process of how ethanol kills bacteria. In this crucial pandemic, I am looking forward to studying more on ethanol and bacteria, especially exploring how ethanol affects the membrane permeability. The knowledge that I gain from this experiment will allow me to explore further and gain understanding how it affects COVID19 specifically.

Hypothesis:

Since ethanol is a solvent, exposing a membrane to ethanol will increase its permeability. Therefore, I believe that the higher the concentration of ethanol, the more permeable the membrane will be. However, I think that if the concentration of ethanol is too high, then this may affect the beetroot cell membrane which causes the membrane to break down. If this occurs then it would come to the point where there is no effect in this experiment as the ethanol would affect the lipids in the membrane. Eventually, causing more of the red pigment from the betacyanin to leak out from the beetroot. Moreover, I believe that when the beetroot is placed into the 0% concentration of ethanol, there may still be a little leakage of the red pigment from the beetroot cells. The reason for this is because when the beetroot are sliced, the cells are also cut causing damage to the cell membrane and the changing of phospholipid bilayer leading to a little leakage of the betacyanin. In addition, the absorbance of light would also increase when the concentration of ethanol increases. 0% ethanol solution will remain transparent , while the 100% ethanol solution will have the darkest red color. Therefore, the relationship between ethanol concentration and light absorbance will be directly proportional. Hence, as the ethanol concentration increases, the permeability of the plasma membrane will increase accordingly and the transmission of light will decrease as there is an increase of the betacyanin leaking.

Null hypothesis:

There will be no relationship between the concentration of ethanol ($C2H5OH$) and the permeability of the beetroot cell membrane.

Sketch graph of hypothesis:

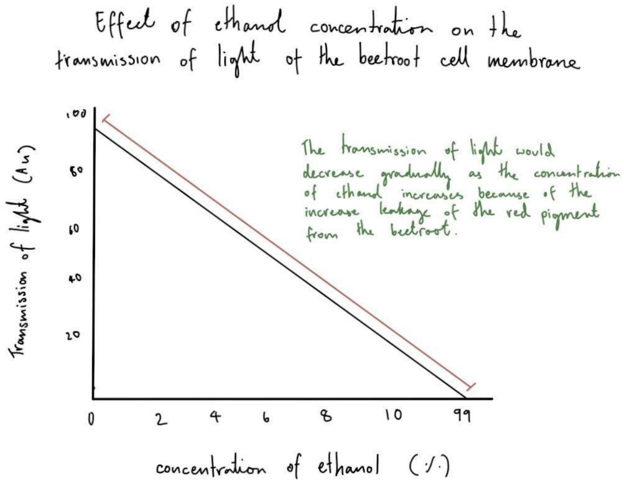

Fig. 1 Author's sketched graph of hypothesis

Variables:

Variables	Description	Method of measuring and their effect
Independent variable	Concentration of ethanol (%) range of 2%-99%	The concentration of ethanol varies with 2%, 4%, 6%, 8%, 10%, 99%. This is modified through dilution. This is carried out by adding the required volume of distilled water where the range of ethanol concentration is 2%-10%. This is because the maximum concentration which could be used is from a 10% ethanol stock solution whereas the minimum is 2%. The distilled water is used for control which is considered as a 0% ethanol concentration.
Dependent variable	Permeability of the beetroot cell membrane which is inferred from light absorbance, transmission of light (Au)	The permeability of the beetroot membrane is measured from the leakage of the betacyanin in the beetroot. The permeability increases as the ethanol concentration increases but the transmission light decreases. This is then put into the colorimeter to determine the transmission of light passing through the ethanol with different concentrations.

Controlled variables:	**Method of measuring and their effect**
Size and type of beetroot (cm)	The beetroot are sliced into identical shapes where only the middle part is used. The thickness and size of beetroot affects the time taken for the ethanol solution to reach the cell plasma membrane. Thus, the size and thickness of beetroot must all be the same for the experiment to be reliable. Moreover, it is essential that the type of beetroot used must be the same since they have different structures even though they are somehow similar. All beetroots used in this experiment are checked with a ruler to make sure that they are all the same size. The ruler used to measure all beetroots must be the same to reduce errors in the measurement.
Volume of ethanol solution (cm^3)	Syringes are used to measure the volume of ethanol solution for accuracy. Equal volume of the total solution was prepared for all trials. It is necessary to keep the volume of ethanol solution constant throughout the experiment so that the effect of ethanol reaching the membrane of the beetroot is all the same and that the experiment is reliable. Also, to ensure that all test tubes, trials contain the same number of moles which can later on be used for calculation if necessary. The volume of ethanol solution used for all trials is 5mL.
Size of cuvette from the colorimeter	Not only the size of cuvette are used for all trials but the type of the cuvette is the same since different types of cuvette are made with different materials which may affect the experiment when using the colorimeter as they perform differently. The cuvette was calibrated at the beginning.
Time	The time taken when the beetroots are placed into the ethanol solution are all the same at 10 minutes. The duration is kept constant to ensure that the data is not invalid. As soon as the beetroot touches the solution, the stopwatch starts immediately for all trails. Trials were simultaneously stopped by taking out the beetroot pieces at the same time. The time taken would affect how much the ethanol solution interacts with the cell membrane. If the duration is short then the ethanol solution would not fully interact with the membrane but if the duration is too long then the solution would have longer time to react with the beetroot cell membrane which may be unreliable as the time is inconsistent.
Temperature	The experiment was conducted in the room at a constant temperature, which is approximately at 24°C (+/- 1)
Colour of the light when using the colorimeter	The colour is set to blue transmission light in this experiment. However, there are different colored lights that can appear as it affects the results to be unreliable when using a colorimeter. Before inserting the cuvette, it is necessary to check the colour of the light.

Method of controlled:

Size of beetroot - Using the same ruler to measure (uncertainty of +/- 0.05cm)
Volume of ethanol solution - Pouring the solution into the beaker and measure using a syringe (uncertainty of +/- 0.05cm3)
Size of cuvette - Ruler and using the same cuvette (uncertainty of +/- 0.05cm)
Time - Stopwatch and timer (uncertainty of +/- 1 second)

Sample results table:

Test Tube	Concentration % (+/- 1)	Volume of diluted ethanol (cm^3) (+/- 1)	Volume of distilled water (cm^3) (+/- 1)	Transmission (Au) (+/- 0.1)		
				Trial 1	Trial 2	Trial 3
A	2	1.0	4.0			
B	4	2.0	3.0			
C	6	3.0	2.0			
D	8	4.0	1.0			
E	10	5.0	0.0			
F	99	5.0	0.0			

Fig. 2 Sample results table

Environmental consideration:

In the experiment, the ingredients used are potentially harmless to the environment. The beetroots are organic products, causing no harm to the environment. The data are not recorded on paper but instead recorded online to minimize the waste production. The leftover beetroots are used in other group's experiments in order to reduce food waste. The solutions are emptied in the water system but it causes no harm to the environment.

Materials:

1) 5 of $10cm^3$ test tube
2) 3 of 100mL beaker
3) 5 pieces of beetroot ($1cm^3$ for width and length with the thickness of $0.5cm^3$)
4) $15cm^3$ of ethanol
5) $10cm^3$ of distilled water
6) Colorimeter
7) Cutting board
8) Test tube rack
9) Distilled water
10) Pipette
11) Timer
12) Forcep
13) Vegetable knife or scalpel
14) Ruler
15) Petri dish
16) 3 Syringe
17) Glass pen

Diagram:

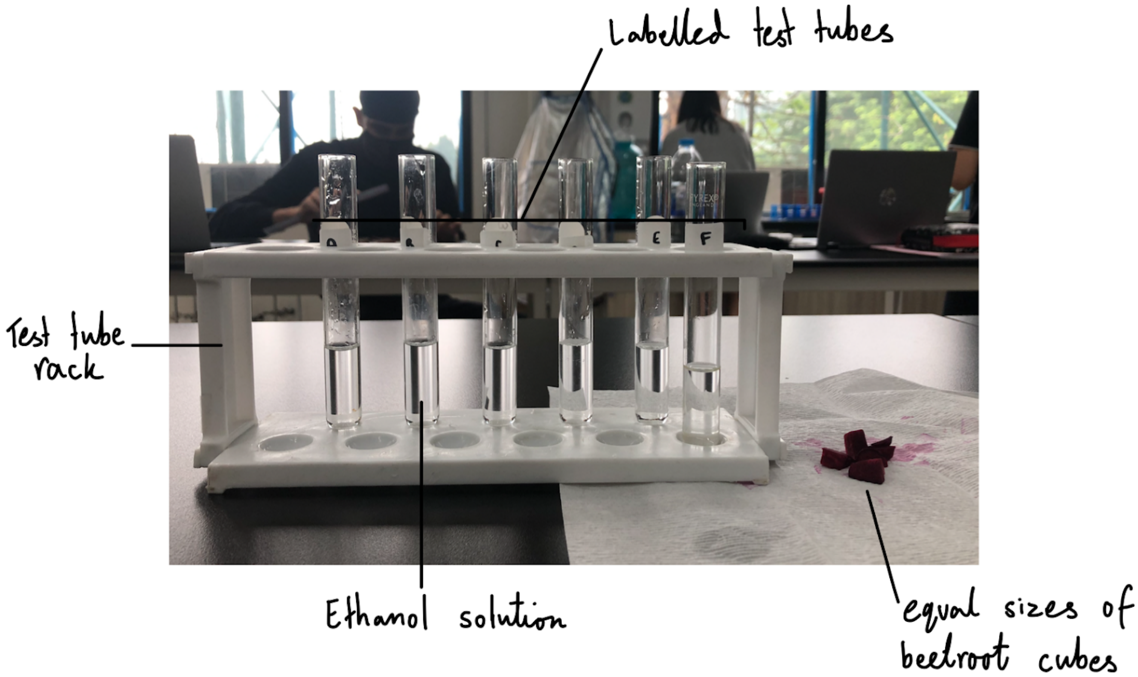

Fig. 3 Labelled diagram of the actual experiment

Method:

1) Use a vegetable knife or scalpel, cut the beetroots into 5 small cubes with an equal measurement of $1cm^3$ for both width and length on a cutting board. Use a ruler to get an exact measurement.
2) Rinse the beetroot pieces with distilled water several times and immediately dry it using paper towels to remove pigment released during the cutting process.
3) Use a pen to label the test tube as A,B, C, D, E, and F. Place all the labeled test tubes in a test tube rack.
4) Pour the 10% ethanol into a $100cm^3$ beaker. Designated a syringe for this beaker, syringe 1. Pour distilled water into another $100cm^3$ beaker. Designated a syringe for this beaker, syringe 2.
5) Use a syringe 1, add $1cm^3$ of 10% ethanol in a $10cm^3$ test tube A. Add $4cm^3$ of distilled water into the test tube using syringe2. Place it in a test tube rack.
6) Use a syringe 1, add $2cm^3$ of 10% ethanol in a $10cm^3$ test tube B. Add $3cm^3$ of distilled water into the test tube using syringe 2. Place it in a test tube rack.
7) Use a syringe 1, add $3cm^3$ of 10% ethanol in $10cm^3$ test tube C. Add $2cm^3$ of distilled water into the test tube using syringe 2. Place it in a test tube rack.
8) Use a syringe 1, add $4cm^3$ of 10% ethanol in $10cm^3$ test tube D. Add $1cm^3$ of distilled water into the test tube using syringe 2. Place it in a test tube rack.
9) Use a syringe 1, add $5cm^3$ of 10% ethanol $10cm^3$ test tube E. Do not add distilled water. Place it in a test tube rack.
10) Pour the 99% ethanol into a $100cm^3$ beaker and designate a syringe number 3.
11) Use syringe 3, add $5cm^3$ of 99% ethanol $10cm^3$ test tube F. Do not add distilled water. Place it in a test tube rack.
12) Set a timer to ten minutes.
13) Use a forcep to place a beetroot cube to a test tube.
14) Immediately start a timer once the beetroot comes into contact with the solution.
15) Once the ten minutes is over, immediately use a forcep to remove the beetroot and place it in a petri dish.
16) Calibrate the colorimeter zero using a cuvette with distilled water.
17) Pour the solution into the cuvette. Use the colorimeter to measure the color intensity of betacyanin in the ethanol solution by using a blue transmission light. Record the data.
18) Repeat steps 8-12 two more times for the same concentration of ethanol. Record the data.
19) Repeat steps 8-14 four more times for the test tube with 4%,6%,8%, and 10% ethanol and record the data.
20) Dispose of the ethanol solution properly. Rinse all of the materials with distilled water. Using a napkin, clean the materials.

Health and safety:

Hazards	Effects	Safety precautions
Scalpel or vegetable knife	They have sharp edges which may cause injury to the skin. Can also harm others.	Cut away from the body and away from other students. Do not use excessive force when working with sharp instruments, not only scalpel. Try to hold the beetroot still to avoid accidentally slicing your own finger. Do not leave blades out after use.
Glassware	Glasswares such as test tubes are fragile causing them to break easily. They have very sharp edges when they're broken, this may cause skin injury.	To avoid breaking the glass, it is essential to handle it with care. Do not put it near the edge of the table so that it won't fall to the ground. If it is broken, it is important to use protective gloves to collect the broken glass and not bare hands. It is also important to plan your procedure before starting the experiment.
Ethanol	Ethanol solution can cause skin irritation and serious eye irritation. May damage to organs. They are also flammable.	Keep the ethanol solution away from heat/sparks. Wear safety goggles and avoid touching it with bare hands if possible.

Results and data collection:

Test Tube	Concentration % (+/- 1)	Volume of diluted ethanol (cm^3) (+/- 1)	Volume of distilled water (cm^3) (+/- 1)	Transmission (Au) (+/- 0.1)		
				Trial 1	Trial 2	Trial 3
A	2	1.0	4.0	81.3	86.0	48.4
B	4	2.0	3.0	89.4	87.1	47.3
C	6	3.0	2.0	79.2	84.1	43.4
D	8	4.0	1.0	39.4	37.3	36.9
E	10	5.0	0.0	46.2	43.3	50.4
F	99	5.0	0.0	7.8	9.7	27.1

Fig. 3 Results table

Processed data:

The average transmission of light is calculated for each concentration of ethanol. By calculating the average value, this will give only one answer from a group of results. The standard deviation is also useful when doing this kind of experiment to indicate the dispersion of the data collection. The higher the standard deviation, the lower the reliability since the data is spread with a large range. Whereas the lower the standard deviation, the higher the reliability since the data is less spread.

Test Tube	Concentration % (+/- 1)	Transmission (Au) (+/- 0.1)			Standard deviation
		Trial 1	Trial 2	Trial 3	
A	2	81.3	86	48.4	20.48682503
B	4	89.4	87.1	47.3	23.67044571
C	6	79.2	84.1	43.4	22.2191359
D	8	39.4	37.3	36.9	1.342882472
E	10	46.2	43.3	50.4	3.569780572
F	99	7.8	9.7	27.1	10.63688551

Fig. 4 Standard deviation

The standard deviation is calculated in google sheets. To conclude, test tube B has the highest standard deviation at 23.7 and test tube D has the lowest standard deviation at 1.3. Meaning that test tube B is the least reliable whereas test tube D is the most reliable.

Calculations:

Formula to calculate the mean average: sum of all trials ÷ number of trials

Test Tube	Concentration % (+/- 1)	Transmission (Au) (+/- 0.1)			Calculation	Average
		Trial 1	Trial 2	Trial 3		
A	2	81.3	86.0	48.4	(81.3+86+48.4) ÷ 3 = 71.9	71.9
B	4	89.4	87.1	47.3	(89.4+87.1+47.3) ÷ 3 = 74.6	74.6
C	6	79.2	84.1	43.4	(79.2+84.1+43.4) ÷ 3 = 68.9	68.9
D	8	39.4	37.3	36.9	(39.4+37.3+36.9) ÷ 3 = 37.87	37.9
E	10	46.2	43.3	50.4	(46.2+43.3+50.4) ÷ 3 = 46.63	46.6
F	99	7.8	9.7	27.1	(7.8+9.7+27.1) ÷ 3 = 14.87	14.9

Fig. 5 Mean average calculation

Qualitative data:

The higher the ethanol concentration solution, the redder and darker the colour of the solution gets. This is due to the increase in leakage of the betacyanin from the beetroot. The solution with 2% concentration has a slight pink colour whereas the highest concentration solution with 99% has a dark purple colour.

Results graph:

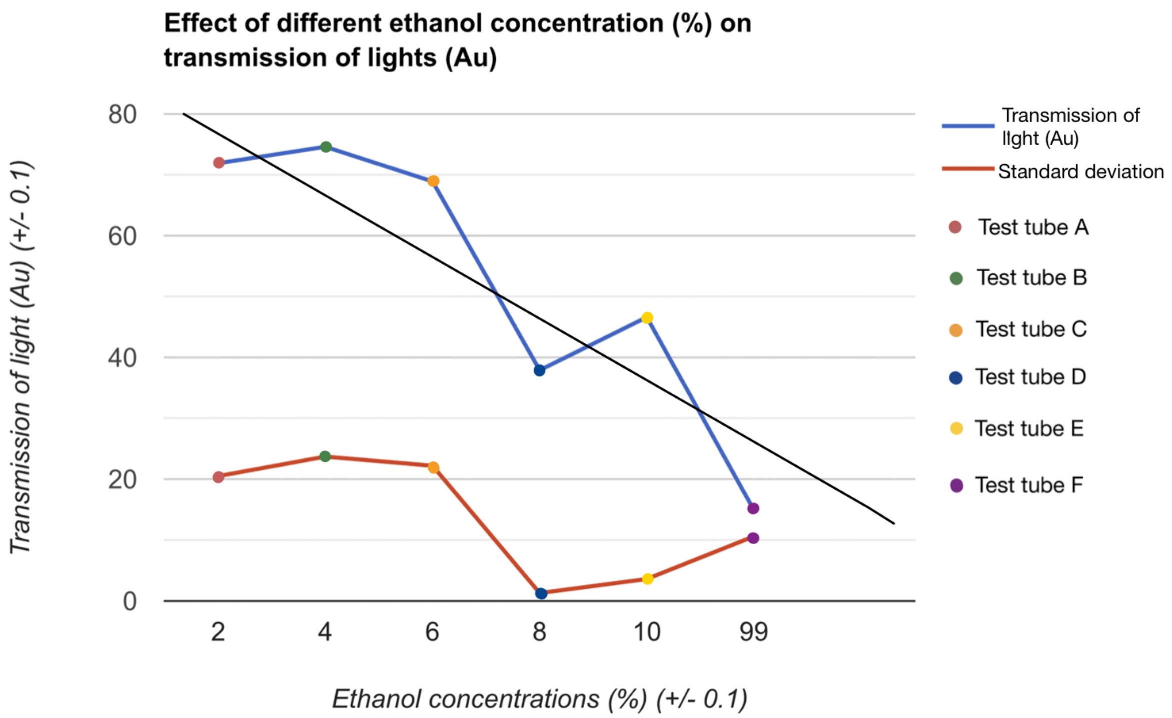

Fig. 6 Transmission of light on ethanol concentrations graph

In an overall trend, the graph shows a strong negative correlation. Demonstrating that the higher the ethanol concentration, the lower the transmission of light. The standard deviation shows the reliability from the results. This illustrates that test tube B has the highest standard deviation meaning that it is the least reliable. While test tube D has the lowest standard deviation meaning that it is the most reliable. If arranged correctly from lowest to highest standard deviation it would be test tubes D, E, F, A, C and B. Where B has the highest error and D with the lowest error.

A Statistical test which is known as T-test is done in google sheets which is used in hypothesis testing. It is used to determine whether a predictor variable has a statistically significant relationship with an outcome variable. The T-test for this experiment is 0.29 from trial 1 and 2, which is the p value or the probability value. This is the probability that the difference we see is purely due to chance. In this case, the t-test is a 30 percent probability it was due to chance. This concludes that the averages are not significantly different. In addition, Spearman's rank is also used to measure the strength and direction of association between two ranked variables. This is calculated using the website socscistatistics. The result is -0.6, which means that there is a negative correlation.

Conclusion:

Since the purpose of the experiment is to see the effect of the ethanol concentration on the beetroot's cell membrane permeability, it is concluded that the higher the ethanol concentration, the more permeable the member becomes. This is supported with the results from the graph illustrating that the increase in ethanol concentration reduces the transmission of light or the light absorbance. By having less transmission of light, it proves that the beetroot's cell membrane is more permeable. This strongly supports the hypothesis. At higher concentrations, ethanol interacts with the beetroot's cell membrane reducing the bilayer stability as well as breaking down the phospholipids bilayer barrier properties, causing an increased permeability. With higher ethanol concentrations, more of the red pigment is leaked from the vacuole, tonoplast as proven by the transmission of light decrease. More of the red pigment, betacyanin in the beetroot would leak as the lipids control the substances that enter and leave the plasma membrane. When the red pigment in the solution increases as ethanol concentration increases, this shows that it absorbs more light. This is supported by the results table when the lowest with 2% of ethanol concentration gives 71.9 Au transmission of light whereas the highest with 99% ethanol concentration gives only 14.9 Au.

The overall result may not be perfect where test tube E, 10% ethanol concentration seems to be out of track with unexpected results; however, the rest gives the same pattern. The reasons for this unexpected result may be because of the temperature of the room which may change throughout the experiment or the size of beetroot which might not be the same as others as it may have not been cut accurately. The results overall strongly support the hypothesis. The standard deviation helps prove the experiment to show how reliable the results are. The strong negative correlation of the graph between the transmission of light and the concentration of ethanol strongly proves the hypothesis where it is directly proportional. The fact that the ethanol solution reaches the beetroot plasma membrane by diffusion, this damages the tonoplast causing the pigment to leak out into the surrounding, making the solution turn red. Ethanol disrupts the physical structure of cell membranes. Because the membrane is selectively permeable to molecules and ions, once they're damaged there are no longer molecule pumps which results in the instability of molecules leading to killing the cell due to its starvation for ATP. To conclude, as the concentration of ethanol increases, the permeability of the beetroot 's membrane increases as there is an increased leakage of the red pigment, betacyanin, from the beetroot.

Evaluation:

The overall experiment is justifiable mainly because of the reliable 3 trials obtained. By having multiple trials, it makes the data a lot more consistent as well as reliable. There are less mistakes by doing this, we can also make sure that our results are not altered by random events and are able to detect errors a lot easier. The fact that we use a colorimeter in this experiment allows us to accurately measure the absorbance and transmission of light from a solution that we placed in the cuvette. This reduces the time of us trying to calculate the transmission of light as it is a lot faster and convenient as well as more reliable. A colorimeter does not require an experienced person to handle it. Moreover, the strength from this experiment is that there is a wide range of ethanol concentrations used in the experiment meaning that it provides more information and concludes the experiment better as we can see the pattern clearly. From the result: the redder the solution, the higher the ethanol concentration.

However, there are some weaknesses in the experiment including the delay in starting the stopwatch which is a human error. This leads to inaccurate data and an unreliable result. This delay affects the time for the ethanol concentration to reach the beetroot membrane. To improve this, one person should be responsible for timing all the solutions so that the results can be consistent. In addition, the size of the beetroots may be inconsistent since it may affect the time of ethanol reaching the beetroot's membrane causing an unreliable result. If the beetroot is too small, then the surface area would reduce, affecting the rate of diffusion. There are some results that are unexpected and do not follow the pattern such as test tube B where in trial 1 the transmission of light is 89.4 au, trial 2 is 87.1 au but trial 3 is 47.3 au. Trial 3 result is different to the first 2 trials; this may be because of the size of beetroots which are not consistent as it affects the permeability of the beetroot's membrane causing high uncertainties. This can be improved by using a tool such as the cork borer and use only the middle part from each strand so that all pieces are approximately the same. Moreover, the beetroot pieces used were not completely washed causing some of the beetroot pieces to have red pigment leaked out and affect the experiment when placed in the solution. This can be improved by washing the beetroot 2 times per piece and using tissue to absorb all the excess water.

For future experiments, other variables can be used to study the effect of alcohol on the beetroot's cell membrane permeability such as temperature by using water baths to control the variable. Different products other than beetroot can also be used in the experiment to see the differences between the two results.

References:

Membrane permeability, Biology Libretexts, 15 August 2020
https://bio.libretexts.org/Bookshelves/Cell_and_Molecular_Biology/Book%3A_Cells_-_Molecules_and_Mechanisms_(Wong)/04%3A_Membranes_-_Structure_Properties_and_Function/4.02%3A_Membrane_Permeability

Membrane structure, Bioninja
https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/13-membrane-structure/

Biology - Cell membranes, University of Birmingham
https://www.birmingham.ac.uk/teachers/study-resources/stem/biology/stem-legacy-membranes.aspx

Advantages and disadvantages of colorimetry, Chrominfo, 11 September 2019
https://chrominfo.blogspot.com/2019/09/advantages-and-disadvantages-of_11.html

Biology IB+, internal assessment
https://sites.google.com/standrews.ac.th/bio/internal-assessment