IB Extended Essay

Biology

Effect of different concentrations (%10, %30, %50, %70, %90) Zeylanicum extracted with ethanol on non-pathogenic Escherichia coli (E. coli) measured by inhibition zones using disc diffusion method

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Introduction:

The idea of investigation came up when I got the flu. In schools nursery herbal treatments are used. The school nurse made me a cinnamon tea for my cough. She also said it will reduce the bacteria that night be in my throat. Using a cinnamon tea sparked the idea of measuring antibacterial activity it on non-pathogenic E. Coli Bacteria have some beneficial uses in the human body as but in other cases, bacteria are destructive, causing diseases. E. coli is in the family of Enterobacteriaceae. I struggle with oral wound infections usually and I always gargle my mouth with cinnamon water. This is one of the main targets that I am using E.coli to see the effect of cinnamon on E.coli that causes oral infections.

Herbal sources are highly used as an antibacterial source for many years. Firstly essential oils are concerned as they are used in alternative medicine. Cinnamon is highly used in our school's nursery for treatment so including cinnamon oil is searched. The research 'Antibacterial Effect of Cinnamon (Cinnamomum zeylanicum) Bark on Cinnamon' showed the highest zones of inhibition observed proves the effectiveness of the use of cinnamon for traditional medication and confirms that it can be used as an antibacterial agent especially at increased concentrations. Also, Cinnamon serves as a pointer for pharmaceutical industries in producing the most effective drugs from plant sources (Abdulrasheed, 19). Many researchers have proved the antifungal and antibacterial effects of cinnamon, which can cause skin and oral infections and food-borne bacteria. Cinnamon has been used as an anti-inflammatory agent, an anti-termite agent, nematicide, a mosquito larvicide, an insecticide, an antifungal agent, and an anticancer agent. Traditionally, cinnamon has also been used as a tooth powder to treat toothache, dental problems, oral microbiota, and bad breath (Abdalla, 1-2). Due to this knowledge, the cinnamon gargle is found to be effective in using a treatment way on oral infections. The support of research on cinnamon guided the experiment to be done specifically on zeylanicum. The effectiveness of cinnamon concentrations should be changed with a specific range. To be able to that alcoholic exctracts are taken under observation and ethanol has more effect on antibacterial activity on Cinnamum Zeylanicum extracts can provide valuable support for antibacterial therapy. The concentrations of %30 and different concentrated cinnamon ethanol extracts give a scale to identify at what point is the antibacterial activity more efficient. The range for the independent variable is identified by the preliminary experiment that is have done by selecting 2 concentrations of cinnamon and their effect on the bacteria %90 were selected in the preliminary experiment to see the effectiveness of cinnamon solutions. Then in %30, an explicitly seen clear zone is observed around the disc and the ranges started not from %30 but %10. According to studies the research question made up as; What is the effect of different concentrations (%10, %30, %50, %70, %90) of Cinnamomum Zeylanicum extracted with ethanol on non-pathogenic Escherichia coli (E. coli) inhibition zones(±0.01mm) using disc diffusion method formed by incubation at 25°C for 24 hours measured by a digital caliper (0.01mm).

Hypothesis

As the concentration of *Cinnamomum Zeylanicum* extracted with ethanol increases inhibition zones around the discs will increase in diameter.

H0(null): There is no statistically significant effect of different concentrations (%10, %30, %50, %70, %90) of *Cinnamomum Zeylanicum* extracted with ethanol on non-pathogenic *Escherichia coli (E. coli*) inhibition zones(±0.01mm) using disc diffusion method formed by incubation at 25°C for 24 hours measured by a digital caliper (0.01mm).

H1(alternative): There is a statistically significant effect of different concentrations (%10, %30, %50, %70, %90) of Cinnamomum Zeylanicum extracted with ethanol formed by incubation at 25°C for 24 hours will show a

positive correlation with non-pathogenic Escherichia coli (E. coli) inhibition zones (± 0.01mm).

Figure 2: Variables; independent variable, dependent Variable, controlled Variable

Independent variable	Different concentrations of ethanol extracts (%10, % 30, %50, %70, %90) of Cinnamomum zeylanicum made by dilution with distilled water to form a 1000µl volume	
Dependent Variable	The diameter of the E. coli growth inhibition zone (mm) (1± mm) formed over 24 hours of incubation at 25°C, indicated through a decrease of *E.coli* growth , found by measuring the widest diameter of the growth inhibition zone with a digital caliper. (±0.01mm)	
Controlled Variable	**Significance**	**Method of Control**
The source of *E.coli* stock solution	The stock solution of *E.coli* culture on nutrient broth and kept sam efor each to minimize the possibility of changes in the concentration of *E. coli* on different agar plates. In case of any errors in the formation of the stock solution on all plates, so it is still allowed to compare of trails.	The *E.coli* samples were diluted with 0.9% saline solution to stop the bacterial growth after incubation. 10.00 µl placed to each agar plate from the same stock solution.
The OD of the cultured *E. coli*	The OD (optical density) of the cultured colony was measured with the spectrophotometer antimicrobial population was confirmed to be 0.0063Au (0.5 McFarland standard).	The OD (optical density) of the cultured *E.coli* colony is diluted with distilled water to confirm the antimicrobial population to be 0.0063Au.
The source of Cinnamon	Due to biological diversification, different cinnamon sources can result in different rates of antibacterial activity. Using the same stock solution confirms the concentrations prepared under the same circumstances.	100 grams of Cinnamon powder and 300mL of ethanol used to prepare the stock solution. After five days sample was evaporated and a thick stock solution is obtained.
The source of agar	To control the nutrients in each agar plate were as any errors in creation of the agar existed in all agar plates.	Using the same agar stock solution and pouring 4mm to all agar plates, to kept the nutrient same for all plates.
The incubation time of the bacteria	To get sure the *E.coli* cultures grow at the same amount of time, allowing comparison of the different concentrations of cinnamon ample extracted with ethanol on *E.coli* growth inhibition.	All petri dishes inhubated for 1440(±10min)
The temperature of incubation	The rate of chemical reaction changes up to temperature. So the rate of E. Coli replication changes t different temperatures. All trails are incubated at the same temperature provides the variations in *E.coli* growth caused by different concentrations of cinnamon sample extracted with ethanol.	All Petri dishes incubated at the same incubator at the same time at 25°C for 1440(±10min)
The distance between each weal	If some discs are placed closer to each other the concentrations of liquids diffusing from the dics into the agar may be different so it could influence the final results.	Petri dish is divided into four quadrants and each disc is placed on the middle of the different quadrants away from each other.
The pipettes and vortex used	Any error that could cause by the defectivity of the devices is eliminated by using the same equipment for all.	The same equipment (electronic pipette, wire loop) is sterilized and used for each trial and concentration.
using sterilized materials.	To reduce the effect of other microorganisms infecting the trails.	Materials are sterilized through autoclave 121 ^{0}C for 20 mins. and all working places are sterilized with 100% ethanol. In each step, a bunsen burner used for sterilization.
Keeping the pH around 7 of cultured bacteria	To not affect the metabolic process and enzymatic activity of bacteria. For E. coli the pH should be kept around 7.	pH is measured to make sure it is around 7. By adding buffer solution the pH it was kept constant.

Material list and apparatus

- *Escherichia coli* Strain B, lyophilized cells
- Incubator Machine Electromag M 3025 BP incubator
- Ethanol 300cm^3
- Volumetric flask 500cm^3 (±0.15)
- Distilled water
- Cinnamon powder 100g (±0.001)
- Foil paper 30cm
- 5 Plastic bottles 60 cm^3
- Erlenmeyer flask 250cm^3
- Beaker 400 cm^3
- Whatman No.1 filter paper 2
- Electronic scale (±0.001)
- Nutrient broth 80cm^3
- Nutrient agar 1000cm^3
- Volumetric flask 500cm^3 (±0.30)
- Saline solution (NaCl 0.9%) 50cm3
- Water bath 100°C
- Magnetic stirrer
- Whatman No.3 filter paper 25
- Test tubes 3
- Screw cab tube
- Petri dish 6 (8.5 mm) (±0.30)
- Inoculating loop
- Swab sticks 7
- Forceps
- Calorimeter (±0.001)
- Electronic pipette 5- 50µL (±0.6)
- Autoclave
- Electronic calliper (±0.1mm)
- Lighter
- Bunsen burner
- pH meter analogue
- Erlenmeyer flask x 6
- Refrigerator
- Buffer Solution

Safety, Ethical and Environmental Issues

For safety and ethical considerations, the culture of E. coli bacteria is carried out in the laboratory of the local technical university. Bacteria cultured on nutrient broth are stored at 0- 3^{o}C and protected from away from sun light. While the Bunsen burner spreads bacteria, it is always kept on the metal tools used, and they are all sterilized with flame sterilization. The hair is tied up, latex glows, safety goggles and lab coats are always worn during the experiment to prevent form contamination and contact with bacteria. E. coli can easily spread through surface contact. For safety concerns before and after the experiment, hands were washed and disinfected with 100% ethanol. All tools were sterilized before the experiment with 100% ethanol and all tools were in good condition to use such as no broken Erlenmeyer flask is used. All trails are placed at the same time to the petri dish to reduce materials used and damage no environment. All surfaces are disinfected with 100% ethanol and all tools that come into contact with E. coli are sterilized by autoclaving. The Petri dishes were discharged by autoclaving at 121^{0}C for 20 mins.
Methodology

Extraction: Cinnamon powder is measured 100 grams for 300 mL of ethanol. Kept in conical flask closed with foil paper at room temperature (20-22°C) in a dark place for five days. Cinnamon solution filtered with Whatman no1 filter paper. Sample is evaporated with water bath until all the ethanol evaporates. It was evaporated up to 70°C degrees to keep the vaporization point of ethanol constant. The thick sample diluted with distilled water. to form the solutions of; %10, %30, %50, %70, %90. By adding 1mL of stock cinnamon solution and 9mL of distilled water for %10. Then samples were stored in refrigerator at 4°C in small and sterile plastic bottles.

Nutrient agar preparation: 28g of nutrient agar powder (CM003B) was being added to 1L of distilled water. Mixed until it dissolves completely. Sterilized by incubating at 121 °C for 15 mins. pH is measured with analogue pH meter make sure it is around 7.2-7.4.

Bacteria Isolate: *E. coli* sample is cultured on 30 mL nutrient broth or 24 hours. The *E. coli* samples were diluted with 0.9% saline solution to stop the bacterial growth after incubation. The optical density is measured with a spectrophotometer to be 0.063 to achieve the standardized colony. A sterile cotton swab dip into *E. coli* suspension that is cultured on nutrient broth in tube. Excess inoculum removed by lightly pressing the swab against the tube wall at a level above that of the liquid. The agar was inoculated by streaking with the swab containing the inoculum. The rubbing procedure repeated by rotating the plate. To ensure an even distribution of the inoculum the process repeated twice. The *E. coli* sample that is cultured on nutrient agar incubated at 25 °C for 24 hours.

Sub-culturing of Bacteria; A distinct colony is taken from cultured *E. coli.* The inoculating loop is sterilized in the Bunsen burner by putting the loop into the flame until it is red hot. Allowed it to cool. The loop cooled by stabbing it in a clean part of the agar. An isolated colony was picked from the agar plate culture by touching with the loop. On a clean petri dish streaked using the quadrant method. The *E. coli* sample that is cultured on nutrient agar incubated at 25°C for 24 hours.

Plantation: The antibacterial activities of the test samples were measured by the disc diffusion method. The agar was poured into a sterile petri dish on a flat surface to a uniform depth of 4 mm. Waited for the medium to solidify. Subculture colony spread on the agar plate. A cotton swab immersed in a subculture solution.

Antimicrobial Bioassay: Using sterile forceps, the Whatman no.3 filter paper was placed in the 10% cinnamon sample. The excess sample was removed by lightly pressing the swab against the tube wall at a level above that of the liquid. Using sterile forceps, the cinnamon disks were placed on the surface of the inoculated and dried plates. Forceps is sterilized with a Bunsen burner each time. Cinnamon discs were pressed down lightly with forceps to ensure complete contact between the disk and the agar surface. The process is repeated 5 times for10% and the other concentrations 30%, 50%, 70%, 90%. The discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 25 °C for 24 hours. After incubation for 24 hours, the zone of inhibition around the discs was measured by a digital calliper.

2. **Results**

Qualitative Data

- The concentrations generally showed circular inhibition zones.
- The concentration of %50 showed more widespread distribution due to *Figure 4: E. coli inhibition zone (Irregularly shaped effect)*
- Smaller inhibition zones observed for the concentrations %10 and %30 compare to other concentrations
- Concentrations of %70 and %90 showed more noticeable difference inhibition with eyesight.

Result

Figure 4: The Effect of Different Concentrations of Cinnamon Extracts and Controls on E. Coli Inhibition Zones (mm) and its Standard Deviation

Substance	Percentage Concentration (%)	E.coli Inhibition Zone (mm) (±1mm)					Mean	
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 5		
Concentrations of ethanol extracts of Cinnamomum zeylanicum	0	0	0	0	0	0	0	0
	10	16,22	15,3	15,54	16,04	16,38	15,89	0,4
	30	17,08	17,3	16,89	17,18	16,96	17,09	0,13
	50	18,78	17,65	18,04	17, 53	18,17	18,03	0,45
	70	19,52	20,18	19,76	20,23	19,57	19,88	0,71
	90	21,67	21,81	20,32	22,03	22,13	21,59	0,65

Figure 5: Method of measurign the E.coli inhibiton zone

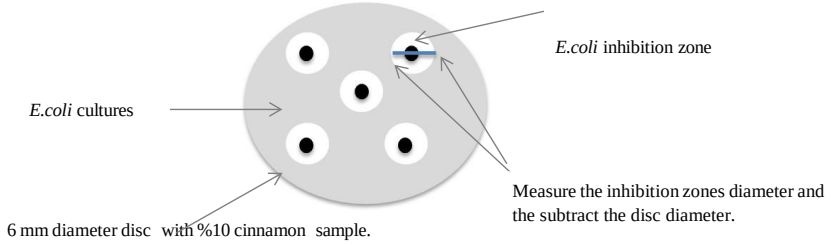


Figure 6: The E.C oli Inhibition Zone areas in totoal (±0.01(±1mm²)) of Different Concentrations of Cinnamon Extracts

Substance	**Percentge concentration (%)**	**E. coli growth inhibition zone on petri dish (±1mm²)**
Concentrations of ethanol extracts of Cinnamomum zeylanicum	0	0
	10	198
	30	227
	50	276
	70	299
	90	365

Sample Calculations

Mean:

$$\overline{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

x_i = The *E. coli* growth inhibition zone of a trail

n = The number of trails of *E. coli* growth inhibition zones measured

1. The *E. coli* growth inhibit zone measurements of all trails added to produce a sum

$16.22 + 15.30 + 15.54 + 16.04 + 16.38 = 79.48$

2. Divided the sum by the number of trials

$$\frac{79.48}{5} = 15.89$$

Standard Deviation: $\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}}$ μ = The mean of all *E. coli* growth inhibition zones of trails N = The number of trails of *E. coli* growth inhibition zones measured	**1.** The mean is found = 16 **2.** For each , the square of its distance to the mean is found $(15.89 - 16.22)^2$ = 0.10 $(15.89 - 15.30)^2 = 0.34$ $(15.89 - 16.04)^2 = 0.02$ $(15.89 - 16.38)^2 = 0.24$ **3.** Results from step 2 is added $0.10 + 0.34 + 0.12 + 0.02 + 0.24 = 0.38$ **4.** Number of trails divided $\frac{0.82}{5} = 0.16$ **5.** Taken into the square root $\sqrt{0.16} = 0.4$ **6.** Rounded to nearest whole number 0.4= 0
***E. coli* inhibition zone** $5.\ (\pi r_a^2)$ $\pi = 3.14$ r = Radius h = The *E. coli* growth zone	**1.** Take the square of the radius of E. coli growth inhibition zone. $\frac{15.89}{2} = 7.95 \rightarrow 7.95^2 = 63.20$ mm **2.** Multiply the square of the radius of E. coli growth inhibition zone with π. 3. $14 \times 63.20 = 198.45$ mm **3.** Multiply with 5 because 5 trails is taken. $198.45 \times 5 = 992.25$
Error $\text{maximum} = \frac{\text{Uncertainty of apparatus used to measure dependent variable}}{\text{variable value}} \times 100\%$ Lowest dependent	$\text{Eroor}_{\hat{x}} = \frac{0.1}{15.89} \times 100 = 0.6\%$

Uncertainty
Processing

$$\frac{absolute\ uncertainty}{measured\ value}\ x\ 100 = percentage\ uncertainty$$

Volumetric flask $1000cm^s \rightarrow \frac{0.30}{1000} \times 100 = 0.03\%$

Volumetric flask $250cm^s \rightarrow \frac{0.15}{250} \times 100 = 0.06\%$

Electronic scaleg $\rightarrow \frac{0.001}{100} \times 100 = 0.001\%$

Electronic scale g → $\frac{0.001}{\quad} \times 100 = 0.003\%$

Calorimeter Au → $\frac{0.001}{0.060} \times 100 = 1.66\%$

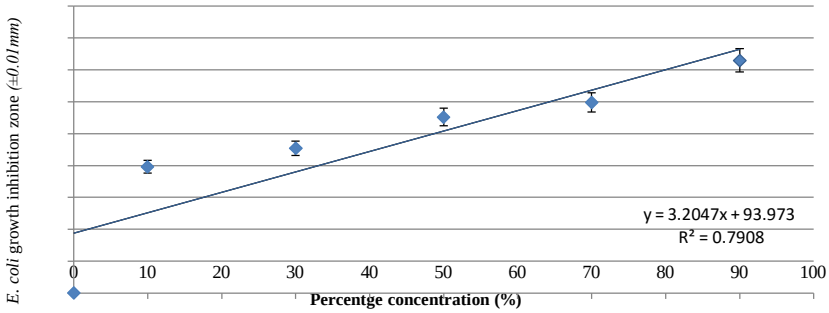
Caliper → $\frac{\quad}{0.1} \times 100 = 0.62\%$

15.89

0.03% % + 0.06% + 0.001% + 0.003% + 1.66% + 0.62% = 2.37%

3. Analysis Statistical Analysis

Figure 7: E. Coli Growth Inhibition Zones (±0.01mm) of Different Concentrations of



Pearson correlation coefficient calculated which, is a measure of linear correlation between two sets of data. Pearson correlation coefficient calculation is used to see the relationship between the different concentrated cinnamon samples and *E. coli* inhibition zone using the data was also appropriate for the use of Pearson correlation coefficient calculation. The hypothesis states the increasing rate of concentrations (%10, %30, %50,

%70, %90) of *Cinnamomum Zeylanicum*, extracted with ethanol, formed by incubation at 25°C for 24 hours will show a positive linear correlation with non-pathogenic *Escherichia coli (E. coli)* inhibition zones(± 0.01mm). The data with increasing rate and the positive linear correlation as it is shown in the figure 7 between the E. coli growth inhibition zone and different concentrated cinnamon samples, extracted with ethanol, the alternative hypothesis is provided to be true. As the Figure 7

a significant increase with concentration.

In each effect on *E. Coli* is observed starting with %10. Along with the antibacterial effect of cinnamon ethanol as alcohol has a significant effect on antibacterial activity compared to aqueous solutions. *Cinnamomum Zeylanicum* extracts with ethanol can provide valuable support for antibacterial therapy (Abdulla, 2584). So E. coli growth inhibition zone Show effect even in the least amounts.

In %50 percent irregularly shaped inhibition zone is observed and it is measured to be less than the other trails of the concentrations. All measured concentration shows variation in trails as figure 5 shows 10%, 50%,70% show variety differentiating around 1mm. All the other concentrations have either one lower or higher inhibition zone compare to their average. For 30% it is less than 0.5mm, however, 90% shows bigger difference with 1.91mm. 90 % shows both a lower (20.32) and a higher (22.13) concentration with a 2.22 mm difference. Also figure 6 shows the standard deviation of 90% which is 2 and has the highest rate.

4. Conclusion

The data with increasing rate and the positive linear correlation as it is shown in figure 7 between the E. coli inhibition zone and different concentrated cinnamon samples, The alternative hypothesis is accepted as the null hypothesis rejected by Pearson correlation coefficient calculation and interpreted result of p value. r value is 0.889295 which shows strong coloration between the variables. Some of the groups' averages consider to be not equal. In other words, the difference between the averages of some groups is big enough to be statistically significant. A smaller p-value which is 0.008853, so null hypothesis (H0) is rejected and H1 hypothesis is supported meaning that there is a statistically significant effect of different concentrations (%10, %30, %50, %70, %90) of *Cinnamomum Zeylanicum* extracted with ethanol on non-pathogenic Escherichia coli (E. coli) inhibition zones.

In each effect on E. Coli is observed starting with %10. Along with the antibacterial effect of cinnamon ethanol as alcohol has a significant effect on antibacterial activity compared to aqueous solutions. *Cinnamomum Zeylanicum* extracts with ethanol can provide valuable support for antibacterial therapy (Abdulla, 2584). So E. coli growth inhibition zone Show effect even in the least amounts. As figure 8 shows all the data lies on the best fit line or shows a significant increase with concentration. So the results obtained a statistically significant positive correlation between different concentrations (%10, %30, %50, %70, %90) of *Cinnamomum Zeylanicum* extracted with ethanol on non-pathogenic *Escherichia coli (E. coli*) growth inhibition zones(±0.01mm) using disc diffusion method formed by incubation at 25°C for 24 hours measured by a digital caliper (0.01mm). The results show *Cinnamomum Zeylanicum* has antibacterial activity on *E. coli*. The antibacterial activity of cinnamon is supported by (Abdulrasheed, 19)(Abdulla, 2584) studies. Since p-value<α, H0 is rejected. The increasing rate of concentrations and the growth inhibition zone of *E. coli* show a correlation. In figure 8 there is a positive linear correlation with increasing concentrations of cinnamon samples so the null hypothesis is rejected.

Also, researches showed that pH and temperature have an important effect on the enzymatic activity of *E. coli.* Cultured E. coli pH is kept around 7.2-7.4. At pH 7 the number of H+ and OH- ions are equal. The optimum temperature is 37 °C for E. coli on culturing. Whereas the studies show that at 25 °C normal growth was observed after 24 hours *on E. coli* (Noor 900). Time is taken 24 hours for 37°C by extending the time the same antibacterial activity aimed to measure. So less time wouldn't give the optimum results for *E. coli,* with more time higher inhibition zone results could have been observed or there may not be a big difference as the 37°C is the optimum temperature for *E. coli.*

The antibacterial effect of different concentrated *Cinnamomum Zeylanicum* is measured. The ethanol extraction caused measurable antibacterial activity even in %10 of cinnamon extract. The percentage uncertainty is found as; 2.37% meaning the accuracy of the data is strong. The medicinal use of cinnamon is measured and found to be effective on bacteria's. The experiment aims to see *E. coli* see the effect of cinnamon on *E.coli* that causes oral infections. The positive linear correlation increasing rate results shows the antibacterial effect on *E. coli.*

Evaluation

Figure 8: Strengths and their significance

Strengths	Significance
Taking a control trail on the same *E. coli* culture	Taking a control trail show the effect of the water impregnated filter paper on nutrient agar. No effect was observed so the precision of the concentrated samples is consolidation.
The range of differently concentrated samples (%10, %30, %50, %70, %90)	The wide range of concentrations allowed the precision of the data and supported the conclusion.
Using disc diffusion method	Disc diffusion method supports the research question the inhibition zone is calculated by subtracting it from the *E. coli* growth zone. To calculate the inhibition zone the *E. coli* needed to be cultured evenly. Discs diffusion method allows this distribution on Petri dish surface.
Controlling many variables	Controlled variables are the source of *E. coli* stock solution, the OD of the cultured *E. coli*, the source of Cinnamon, the source of agar, the incubation time of the bacteria, the temperature of incubation, the distance between each well, the pipettes, and vortex used, using sterilized materials and keeping the pH around 7 of cultured bacteria.
Wide range of independent variable	The wide range of independent variables gives a wide range of relevant data. The wide range of data allows the analysis calculations to be made.
Taking strict safety precautions	For safety concerns before and after the experiment, hands were washed and disinfected with %100 ethanol. All tools were sterilized before the experiment with %100 ethanol. Along with these safety concerns, steam sterilization could have been used to be sure of the sterilization of the materials and dispose of the bacteria.
Percentage Uncertainty	The percentage uncertainty is measured 2.37% which shows small value meaning the accuracy of the experiment is strong.

Limitations

Figure 9: Limitations, their effect and suggested improvements.

Limitations	Effect on Results	Improvements
Using magnetic stirrer	The evaporation took a long time and when ethanol evaporated the cinnamon formed a thick solution and it sank to the bottom of the beaker. The magnetic stirrer was not enough the solution was stirred by hand. This affects the evaporation process and the accuracy of the cinnamon solutions.	A rotary evaporator could have been used to evaporate ethanol from the cinnamon sample.
Drops remained in the pipette	Small amounts are used while arranging the absorbance of the bacteria. This could result in an inaccurate absorbance rate and bacterial culture. This directly affects the bacterial culture and inhibition zones of cinnamon extracts.	Sophisticated and accurate measuring electronic pipette should be used
Human error	The same person can conduct all experiments can reduce the error, but it cannot be exterminated.	While mixing solutions, streaking the cotton swab, using the inoculating loop, placing the docs and keeping the time; human reaction time will be slightly different, resulting in a slight decrease in the accuracy of the results.
Disc placement on nutrient agar	Kirby- Baur essay method could have been used. In Kirby- Baur method the discs are placed in agar wells that are opened.	Kirby- Baur essay method could have been used. In Kirby- Baur method the discs are placed in agar wells that are opened.

Extensions

Ethanol samples with varying concentrations as in the experiment (%10, %30, %50, %70, %90) could have been replaced with peppermint. The antibacterial activity of peppermint oil against some Gram-positive and Gram-negative bacterial strains was evaluated in the present research work by agar well diffusion method. It was found that the distilled concentrations of essential oil inhibited the growth of microorganisms (Singh, 322). The proven antibacterial activity can be measured using the same procedure. The evaporation method could have changed as both the ethanol and the peppermint extract will be liquid. The serial dilution method could use to arrange the concentrations of peppermint.

Minimum inhibitory concentrations (MIC) are characterized as the most reduced centralization of an antimicrobial that will repress the noticeable development of a microorganism after overnight brooding, and least bactericidal fixations. As the most reduced centralization of antimicrobial that will forestall the development of a living being after subculture on to anti-infection free media. MIC's can be measured with Micro-dilution using the 96-well micro-titre plate. By preparing serial dilutions of cinnamon extract and then inoculating the discs with the organisms after the incubation. The lowest concentration that inhibited a visible growth of the inhibition zone and that is the MIC value.

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