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Evidence and Potential Antibacterial Mechanism of Chinese Traditional Medicine Compounds for the Development of *E. coli*

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Abstract

Astragalus membranaceus (*huangqi*), *Allium sativum* (*garlic*), *Cinnamomum cassia* (*cinnamon*), and *Dolichos lablab L.* (*white hyacinth bean*) are the traditional Chinese herbs that were used in prescriptions in treating diarrhea caused by bacterial infection. These herbs are relatively safe for use and investigation. This study aimed to investigate the effects of *Astragalus membranaceus*, *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L. on the metabolism of Escherichia coli* (*E. coli*). The growth rate of *E. coli* was monitored under the influence of each herb, revealing that *Astragalus membranaceus* and *Allium sativum* exhibited significant antibacterial activity, whereas *Cinnamomum cassia* and *Dolichos lablab L.* demonstrated moderate inhibitory effects on *E. coli* growth. Further inhibition zone testing allowed for the evaluation of each herb's potency and the number of generations required for *E. coli* to develop resistance. Additionally, the impact of the four herbs on the expression of outer membrane protein A (OmpA) in *E. coli* was examined by using qPCR. The findings revealed that *Astragalus membranaceus* acted as a sustainable bactericide by inhibiting the growth and metabolism of *E. coli* MG1655 through the suppression of OmpA expression. These results suggest that *Astragalus membranaceus* has potential as a natural antimicrobial agent for treating *E. coli* infections.

Keywords

E. coli, Traditional Chinese Herbs, Antibacterial, OmpA

1. Introduction

Escherichia coli (*E. coli*) MG1655, a common strain of bacteria, presents a considerable public health risk as it is associated with a higher risk of acute necro-

tizing pancreatitis and an increase in inflammatory cytokines in the ileum, according to research by Zheng, J., *et al.* [1]. Notably, this strain of bacteria forms an essential component of the intestinal flora, and any imbalances could potentially heighten the risk of intestinal diseases.

Erfan & Marouf [2] discovered that essential oil from cinnamon exhibits a bactericidal effect on *E. coli*, opening a research path into whether common herbs, thought to possess antibacterial properties, genuinely exert such effects. Being natural, these herbs are deemed to have fewer side effects and therefore represent a safer approach. What are selected for testing are *Cinnamomum cassia* (*cinnamon*), *Dolichos lablab L.* (*White hyacinth bean*), *Allium sativum* (*garlic*), and *Astragalus membranaceus* (*Huangqi*), all of which have shown some degree of antibacterial effect in previous studies.

For instance, Erfan & Marouf's study found that cinnamon oils suppressed *E. coli*'s virulence gene expression, leading to bacterial lysis and shrinkage [3]. *Dolichos lablab L.* also demonstrated antimicrobial effects, particularly against *E. coli* [4], and showed anti-inflammatory effects, indicating its potential for treating bacterial inflammation. *Allium sativum* (*garlic*) also showed potential for inhibiting *E. coli* [5] [6] [7], despite its essential oil enhancing *E. coli*'s biofilm formation [8].

Astragalus membranaceus shows promise in mitigating inflammation, particularly in the intestine and colon, by regulating the expression of adhesion molecules on endothelial cells triggered by TNF-α and lipopolysaccharides (LPS) [9]. These findings connect to the Outer Membrane Protein A (OmpA) of *E. coli*, a major source of inflammation through its facilitation of *E. coli*'s tissue adherence [10]. Further, *Astragalus membranaceus* essential oils have demonstrated an antibacterial effect in past studies [11].

E. coli's membrane proteins, particularly the Outer Membrane Protein A (OmpA), play crucial roles in the bacterium's virulence and adhesion to hosts. OmpA proteins are keys to pathogenic functions, including bacterial adhesion, invasion, or intracellular survival, as well as evasion of host defenses or triggering pro- inflammatory cytokine production [12]. Thus, using qPCR allows researchers to explore the relationship between the tested herbs and the gene expression for OmpA. By investigating the impact of herbal mixtures on outer membrane proteins, we gain insights into the mechanism that eradicates *E. coli*, offering potential solutions for food preservation, consumption, and treatment of bacterial infections.

Building upon these findings, the aim of this study is to delve deeper into the antibacterial properties of these four chosen herbs: *Astragalus membranaceus*, *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L.*, specifically in their effects on *E. coli* MG1655. The study is designed to scrutinize their impact on the bacteria's outer membrane protein A (OmpA), a key player in its virulence. By discerning the herbs' potential in regulating OmpA expression, we aim to uncover new understanding and practical solutions for mitigating *E. coli*-related

health risks.

The endeavor aligns with the broader objective of discovering natural, less invasive ways to control harmful bacteria, thereby improving public health. Given the safety profile of these herbs, their potential use could extend to various applications, including food preservation and adjunctive treatments for bacterial infections. We also aspire to contribute to the body of knowledge concerning the antibacterial properties of these herbs and their implications in the context of bacterial resistance, a growing concern in the medical community. Ultimately, this study strives to bridge the gap between traditional botanical wisdom and modern scientific validation.

2. Materials and Methods

2.1. Ethical Approval

This study did not include both animal and human as research subject, so the study does not require ethical approval.

2.2. Cell Preparation

A colony of transformed strain (K-12 MG1655, MiaoLingBio) was obtained and placed in 5 mL LB culture, containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl. The cells were grown in a shaker at 37˚C, 200 rpm overnight to produce monoclonal strains. The strain was diluted 1:500 in 30 mL modified LB culture, containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, and 0.1% glucose. Strains were then grown in a shaker at 37˚C, 200 rpm until OD_{600} reached 0.6 - 1.0.

2.3. Growth Rate

The study involves testing the effect of different concentrations of essential oils extracted through the steam distillation method [13] from *Astragalus membranaceus*, *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L.* on the growth rate of *E. coli*. The essential oils were diluted in dimethylsulfoxide (DMSO) to various concentrations (0.04%, 0.16%, 0.64%, and 2.56%) for *Astragalus membranaceus*, *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L.* These concentrations were set based on the previous articles. In the case of *Astragalus membranaceus*, due to a dearth of comprehensive studies, additional concentrations were explored (0.16%, 0.32%, 0.64%, 1.28%, 2.56%, and 5.12%).

For each concentration, 1100 μl was added to 12 sterile 50 ml tubes, with the 13th tube treated with 1100 μl of DMSO and the 14th tube with 1100 μl of LB broth to serve as controls for *E. coli* growth without stimulation. A 300 ml LB broth solution containing 0.1% glycerobacterium was prepared in a conical flask and 20 ml of *E. coli* solution was added to each of the 50 ml tubes. The tubes were then incubated in a shaker set at 37˚C and 200 rpm.

The growth rate of *E. coli* was monitored using a spectrophotometer (UV-2100, Jinghua Technology Instrument) by measuring the absorbance at 600 nm. The

spectrophotometer was preheated for 30 minutes and calibrated using LB broth as the blank. The absorbance rate of the *E. coli* was measured thrice using a 2 ml sample of the mixture from each tube at 12-hour intervals, with the final trial ending at 48 hours.

2.4. Zone of Inhibition Test

Discs from BKMAM were soaked with four herbs' essential oils: *Cinnamomum cassia*, *Dolichos lablab L.*, *Allium sativum Astragalus membranaceus*, distinctively; concentrations were 1%, 5%, 25%, 50% (V/V), diluted with DMSO. Wait for the disc to be completely soaked.

Prepare the petri plate by adding, 200 µl of *E. coli*. Use the glass spreading rod to coat the bacteria evenly. Wait until it is dry.

Place the discs on the area being marked; place 4 discs per plate. Put the petri plates upside down and in the incubator, at 36˚C for 24 hours. Then, measure the diameter of the inhibition zone.

2.5. OmpA Gene Expression Measurement

2.5.1. RNA Extraction

Preparation: Set the concentration to 0.16% (40 µl essential oils + 25 ml *E. coli*-LB mixture) with 6-time intervals (0.5 h, 1 h, 3 h, 6 h, 12 h, 24 h). The step is repeated twice more, each time activating the *E. coli* in different preserved tubes. Centrifuge the *E. coli* samples at 4˚C, 12 rpm; pour the LB mixture into the waste tank with only *E. coli* left in the 1.5 ml centrifuge tube.

Use the Trizol method to extract the RNA. Add 500 µl trizol to the centrifuge tube. Put it on the Vortex oscillator for 30 seconds each, so that it is completely mixed. Stand the solution for five minutes. Flip it up and down 50 times and transfer 100 µl of chloroform to each centrifuge tube. Flip it up and down 50 times. Centrifuge it at 4˚C, 12 rpm for 15 minutes. Prepare new 1.5 ml centrifuge tubes. Gently take it out and transfer the top layer solution to the corresponding new tubes without touching any other layers. Transfer 250 µl of isopropanol to the new tubes. Rest it for 10 minutes then centrifuge it at 4˚C, 12 rpm for 10 minutes. Pour the liquid into the tubes to the waste tank. Add 500 µl the 75% ethanol (75 ml ethanol + 25 ml DEPC water). Centrifuge it at 4˚C, 7.5 rpm for 5 minutes. Pour the liquid into the waste tank, and add 500 µl the 75% ethanol again. Centrifuge it at 4˚C, 7.5 rpm for 5 minutes. Pour the liquid into the waste tank. Put the tubes on the ice and wait for it to dry. Then, add 50 µl of DEPC water. Test the concentration of the RNA by using Nanodrop 2000 (Thermo). Then turn them to the same concentration.

2.5.2. Reverse Transcription

Evo M-ML V Reverse Transcription Premix Kit (AG) was used to form cDNA. 8 µl of RNA are added to 8-strip PCR tubes distinctively with 2 µl of 5X gDNA Clean Reaction Mix. Put the solution in the PCR machine for 42˚C, 2 minutes,

then 4˚C forever. Then, add 4 μl 5X Evo M-ML V RT Reaction Mix and 6 μl RNase-free water to each well. Set the PCR machine to 37˚C for 15 minutes, 85˚C for 5 seconds, and 4˚C forever.

2.5.3. Relative qPCR

A real-time PCR assay was developed based on a newly designed primer from the OmpA gene. Use the SYBR Green Pro Taq HS Premix qPCR Kit (AG). Transfer 12.5 μl of 2X SYBR Green Pro Taq HS Premix, 1 μl forward primer, 1 μl reverse primer, and 8.5 μl ddH2O and 2 μl cDNA to new wells separately.

3. Result

3.1. Growth Rate

As shown in Figure 1, a trend of *E. coli*'s growth under different concentrations of *Astragalis membranaceus* is established. At 0.04% concentration, there is an increase in OD_{600} over time, but the values are higher than the control group and DMSO group, indicating a potential inhibitory effect on bacterial growth. At 0.16%, 0.64%, and 2.56% concentrations, there is a decrease in OD_{600} compared to the control group. Specifically, 0.16% and 2.56% concentrations decrease in the first 12 hours, followed by an increase in the next 12 hours. The 2.56% has continuously decreased after 24 hours. The 0.64% concentration decreased until the 36th hour, then exhibited an increase. Therefore, the data is supportive in suggesting an inhibitory effect or bactericidal effect on bacterial growth.

From Figure 2, a trend of *E. coli*'s growth under different concentrations of *Allium sativum* is established. At 0.04% concentrations, there is an increase in *E. coli*'s concentration over time, but the values are lower than the control group at 24 h, 36 h, and 48 h, suggesting a potential inhibitory effect on bacterial growth. At 0.16% concentration, the OD_{600} values are lower than the control group throughout the entire time period, indicating a bactericidal effect on bacterial growth. The *E. coli* under 0.64% concentration, follows the same growing trend with 0.16%. At 2.56% concentration, there is a decrease in OD_{600} through the first 36h, following an increase between 36 h and 48 h. The concentration falls below 0.16% and 0.64% at the time interval between 24 h and 36 h. Even though there are fluctuations in the bacteria's concentration throughout the time, the values remain lower than the control groups, suggesting an inhibitory and bactericidal effect on bacterial growth.

From Figure 3, a trend of *E. coli*'s growth under different concentrations of *Cinnamomum cassia* is established. At the relatively low concentrations: 0.04% and 0.16%, there is an increase in OD_{600} over time, but the values are lower than the control groups and DMSO group. This indicates a potential inhibitory effect on bacterial growth. At 0.64% concentration, there is an increase in OD_{600} up to 24 h, followed by a decrease at the time between 24 h and 36 h, then a slight increase at 48 h is also exhibited. There might be a more complex interaction between *E. coli* and *Cinnamomum cassia* at this concentration in order to establish

this unpatterned fluctuation through time. At 2.56% concentration, there is a slow increase in OD_{600} over time. There is approximately no increase in the first 12 hours displays with the lowest overall growth, suggesting a strong inhibitory effect on *E. coli*.

From Figure 4, a trend of *E. coli*'s growth under different concentrations of *Dolichos lablab L.* is established. At all concentrations: 0.04%, 0.16%, 0.64%, and 2.56%, there is an increase in OD_{600} over time, but the values are lower than the control group. All concentrations follow a similar trend, which is that the most rapid growth occurs in the first 12 hours, then presents a slight decrease at 24 hours (except 0.04%). Overall, it indicates a potential inhibitory effect on bacterial growth.

Because *Astragalus membranaceus* had the best anti-bacterial effect, a more detailed insight into its data is performed.

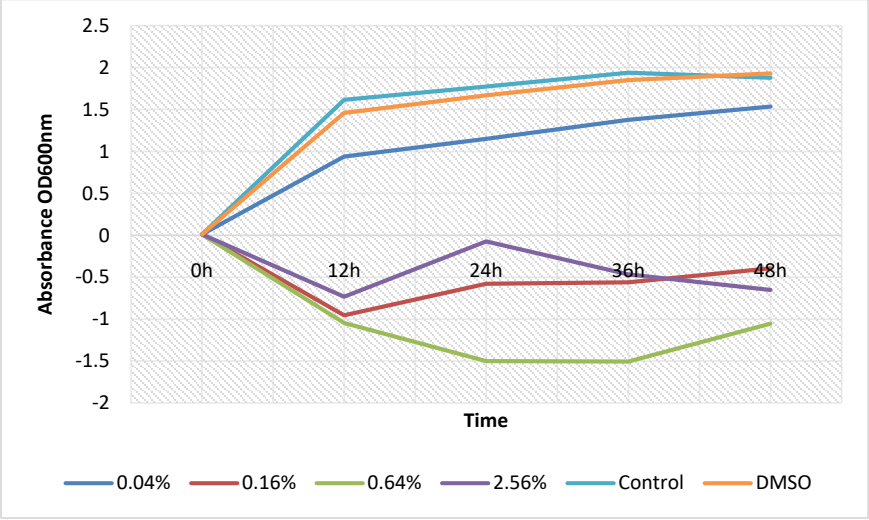

Figure 1. Growth rate of *E. coli* under *Astragalus membranaceus*.

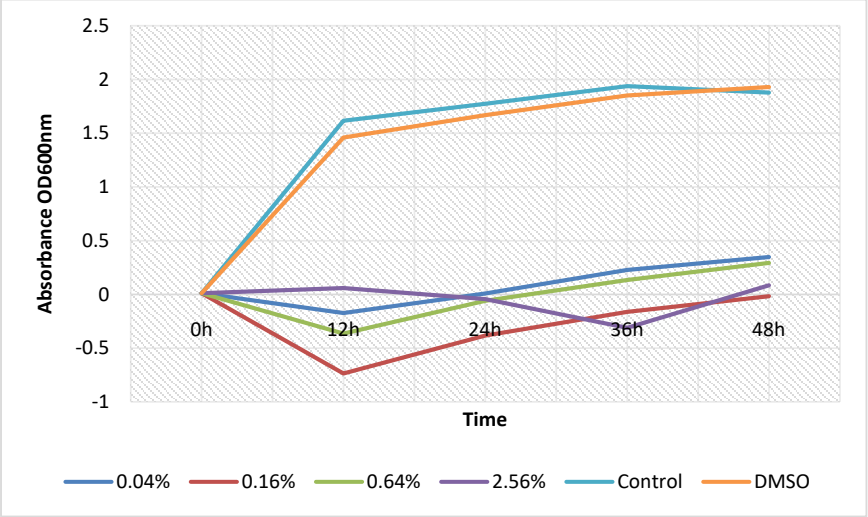

Figure 2. Growth rate of *E. coli* under *Allium sativum*.

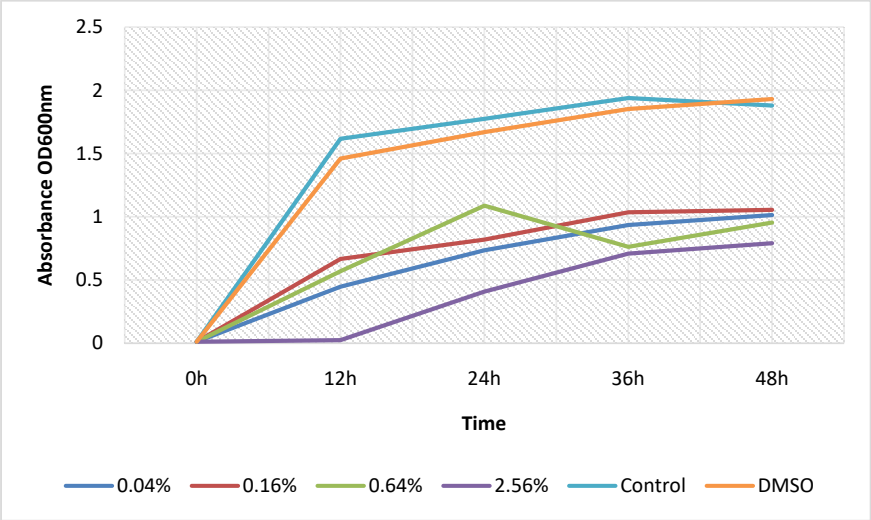

Figure 3. Growth rate of *E. coli* under *Cinnamomum cassia*.

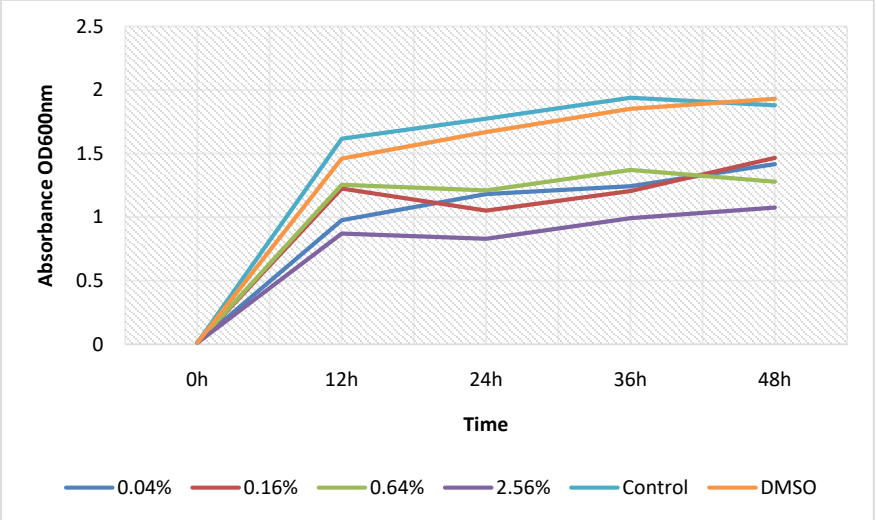

Figure 4. Growth rate of *E. coli* under *Dolichos lablab L.*

From **Figure 5**, the growth rate of *E. coli* exhibits a decline with an increase in concentration of *Astragalus membranaceus*. The group with DMSO and LB only group show approximately no difference. The bactericidal effect has displayed from 0.64% to 5.12%. Anti-bacterial effect had shown through all time intervals, yet, with the strongest effect at the time interval between 0 hours to 6 hours; the anti-bacterial effect has generally increased along the concentration gradient of the essential oil The antibacterial effect of *A. membranaceus* with a concentration of 0.16% and 0.32% had decrease as time pass, but still able exhibit viable inhibiting power. The growth rate of *E. coli* with only LB broth and DMSO follows the logarithm model, while the ones with *A. membranaceus* essential oil of concentration of 0.16%, 0.32%, and 0.64% added follows an approximate linear pattern. For *E. coli* with *A. membranaceus* in 1.28%, 2.56%, and 5.12% concentration shows limited growth that makes the line fluctuate around the zero line.

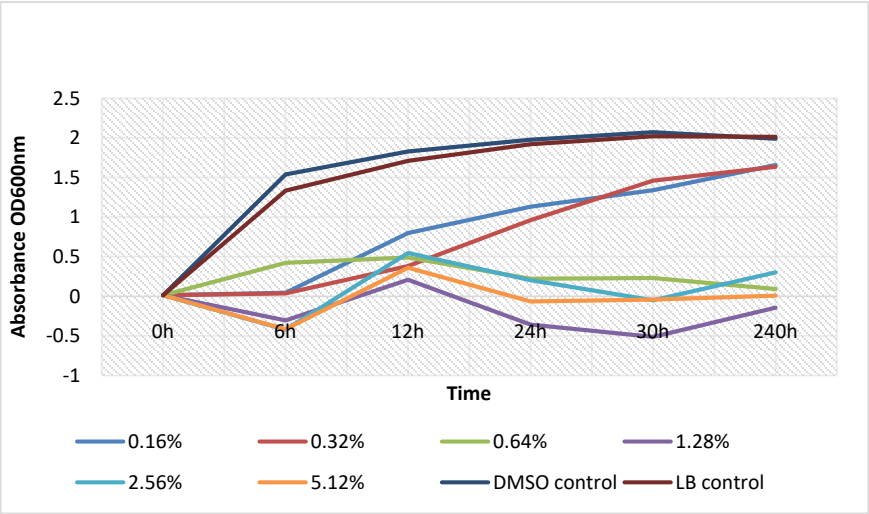

Figure 5. Growth rate of *E. coli* under more concentration of *Astragalus membranaceus*.

From the bar chart (**Figure 6**), the difference between growth rate under different herbs is discovered. In the 0 hour, *E. coli* in all concentrations is the same in density. In the 6 hours, *E. coli* under all concentrations exhibit an inhibition effect or even bactericidal effect. Yet, 0.64% was not as effective as other concentrations. Rest follows a trend of increase in inhibition power as an increase in concentration. In the 12 hours, 0.16% and 0.32% shows a more rapid growth compared to other concentrations, and 0.64% shows almost no increase in *E. coli* density. The *E. coli* in the concentrations of 1.28%, 2.56%, and 5.12% also illustrate a growth, though still having an inhibition effect. In the 24 hours, *E. coli* in 0.16% and 0.32% have continued to grow while others show an increasing bactericidal effect. In the 30 hours, the density of *E. coli* in 0.16% and 0.32% both increased, but faster in 0.32%. Other concentrations continuously depicted the bactericidal effect. In the 240 hours, 0.16% and 0.32% both increased, but still haven't reached the growth rate of LB only medium and medium with DMSO. Only 2.56% shows a slight increase in growth.

In the experiment of growth rate, we are able to conduct that *Astragalus membranaceus* has the best inhibitory and bactericidal effect, while *Allium sativum* is the second. Even though the other two Chinese traditional herbs also establish an inhibitory effect, it is comparatively weak compare to the *Astragalus membranaceus* and *Allium sativum.*

3.2. Zone of Inhibition

Based on the one-way ANOVA test with the data provided in **Figure 7**, we can compare the performance of the four herbs: *Astragalus membranaceus* (Group 1), *Allium sativum* (Group 2), *Cinnamomum cassia* (Group 3), and *Dolichos lablab L.* (Group 4). The table presents the sum, mean, and variance for each herb. *Astragalus membranaceus* has the highest sum (5.5) and mean (1.375), indicating the strongest overall effect among the four herbs. In contrast, *Allium sativum* has a

lower sum (3.1) and mean (0.775), while *Cinnamomum cassia* and *Dolichos lablab L.* have even lower values, with sums of 0.9 and 1, and means of 0.225 and 0.25, respectively.

The F-value (8.278578) is statistically significant with a p-value of 0.00033, which is less than the standard significance threshold of 0.05. This indicates that there is a significant difference between the groups. The F crit value (2.772853) is lower than the F-value, further supporting the significant difference among the groups.

In conclusion, the analysis demonstrates that *Astragalus membranaceus* has the strongest effect among the four herbs, followed by *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L.* This suggests that *Astragalus membranaceus* may be the most effective herb for the desired outcome, while the other herbs are less effective or may require further investigation.

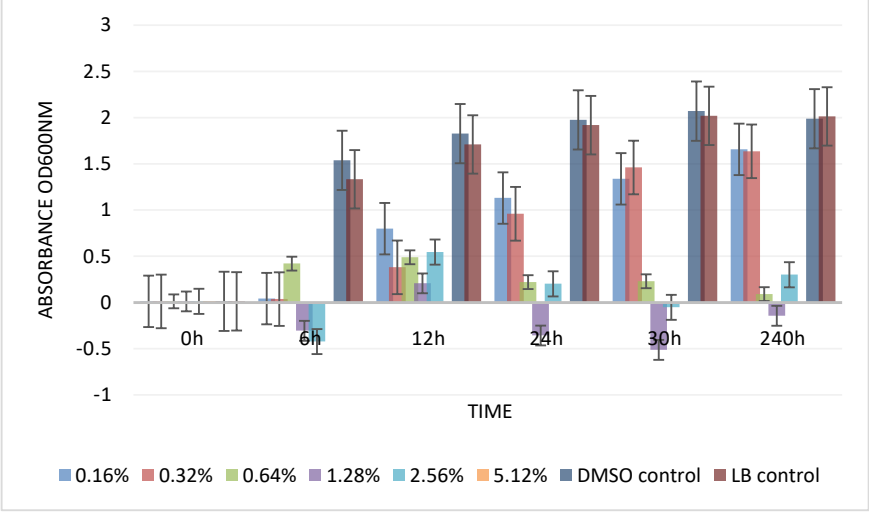

Figure 6. Comparison of growth rate of *E. coli* under six concentrations of *Astragalus membranaceus*.

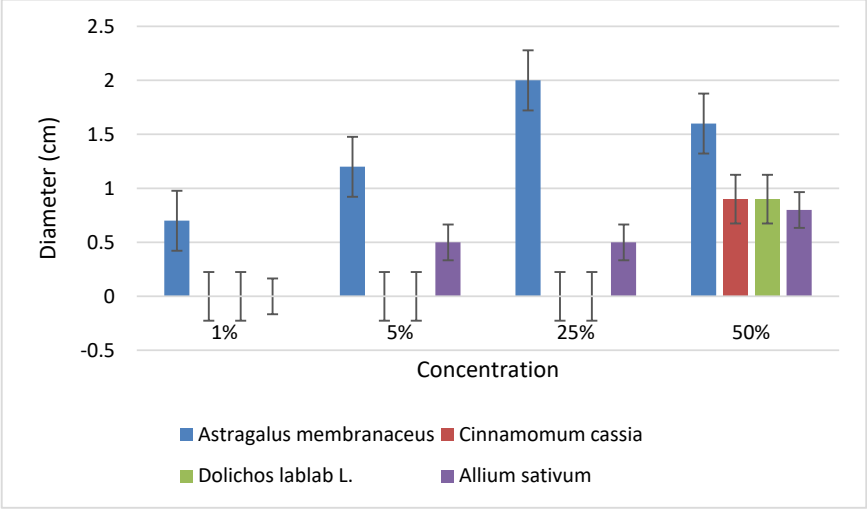

Figure 7. The diameter of clear zone of inhibition in different concentration.

The experiments examining the effects of various concentrations of four different herbs on *E. coli* yielded insightful results. It was observed that ddH_2O and DMSO had no significant impact on the bacteria. In contrast, *Astragalus membranaceus* demonstrated a notable inhibitory effect at all four concentrations tested, with the diameter of the inhibition zones ranging from 1.1 cm to 1.9 cm. This suggests that *Astragalus membranaceus* exhibits a strong antibacterial effect and performs best in the duration of inhibition at concentrations between 5% and 50%.

Allium sativum (*garlic*), on the other hand, showed no significant zone of inhibition. The presence of shallow rings around the discs might indicate an initial inhibitory effect, but the bacteria soon developed resistance. Similarly, *Cinnamomum cassia* (*cinnamon*) displayed a modest zone of inhibition at 50% concentration (0.8 cm), but other concentrations did not show significant results. *Dolichos lablab L.* (*white hyacinth bean*) exhibited only a shallow ring of inhibition (1.0 cm) at 50% concentration, with no visible antibacterial effect at other concentrations.

In conclusion, the experiments reveal that *Astragalus membranaceus* has the strongest and most consistent antibacterial effect against E. coli among the herbs tested, while *Cinnamomum cassia* demonstrates moderate anti-resistance performance at 50% concentration. However, both *Allium sativum* and *Dolichos lablab L.* appear to have limited effectiveness and do not exhibit strong antiresistant effects.

3.3. Relative qPCR

Through running the relative qPCR assays, the trend of the expression of the Outer membrane protein A gene (OmpA) under pressure from different herbs is depicted.

Figure 8 indicates that under the pressure of 0.16% of *A. membranaceus* essential oil, the expression of OmpA had a general trend of decrease. When first added, the expression of OmpA significantly increases. Half an hour after the first test, the expression of the OmpA gene drop dramatically. Then, in the later hours the expression of the OmpA gene had decreased gradually until another huge drop in the expression at the 12 hours.

This might indicate that after adding the *A. membranaceus* essential oil, the *E. coli* was facing osmotic stress. Membranolysis might have happened to *E. coli*. An immediate rise in the expression of OmpA serves to produce more OmpA in order to help *E. coli* to survive during the osmotic stress. However, the expression was soon inhibited by *A. membranaceus*, causing the downregulation of OmpA gene and inhibiting the growth of *E. coli*.

Figure 9 illustrates that under the pressure of 0.16% of *A.sativum* essential oil, the expression of OmpA had a general trend of decrease and then increase. The graph shows a downregulation of the OmpA gene in the first 6 hours, then a visible upregulation from 6 to 24 hours.

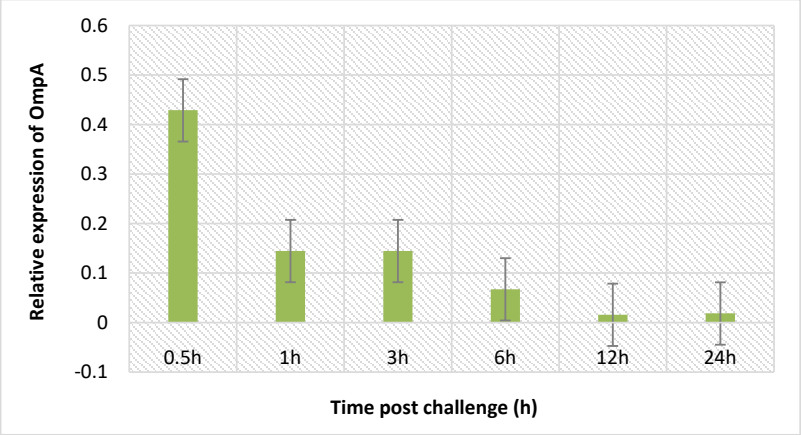

Figure 8. Relative expression of OmpA under *Astragalus membranaceus*.

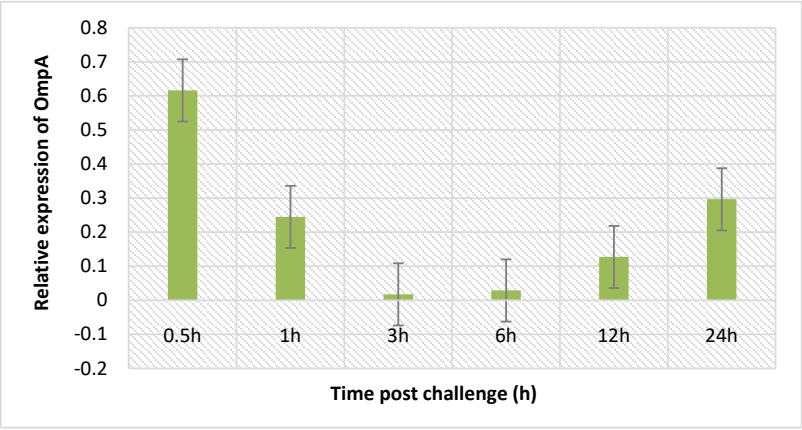

Figure 9. Relative expression of OmpA under *Allium sativum*.

In Figure 10, there is no obvious pattern of the regulation of OmpA gene expression under 0.16% of *Cinnamomum cassia*. It first decreases then increases, reaches a peak at 12 hours, then decreases again. OmpA is expressed in different quantities for regulating *E. coli* in order to survive. The wavy trend shows that even though *E. coli* struggles but it is still able to live in such environment.

Figure 11 indicates that under the pressure of 0.16% of *Dolichos lablab L.* essential oil, the expression of OmpA had a general trend of decrease then increase then decrease. It first decreases from 0.5 hour to 1 hour. Then increases, reaches a peak at 6 hours, then decreases again. OmpA is expressed in different quantities for regulating *E. coli* in order to survive. The wavy trend shows that even though *E. coli* struggles but it is still able to live in such environment.

Compared to condition in *A. membranaceus* and *A. sativum*, the amount of expression of *E. coli*'s OmpA gene under *C. cassia* and *D. lablab L.* is higher. Both OmpA initial expression under *A. membranaceus* and *A. sativum* is less than 1, while under *C. cassia* and *D. lablab L.* is higher than 2. This suggested that the OmpA gene downregulation has occurred visibly in addition with *A. membranaceus* and *A. sativum*, even the initial expression is high compare to itself in different period.

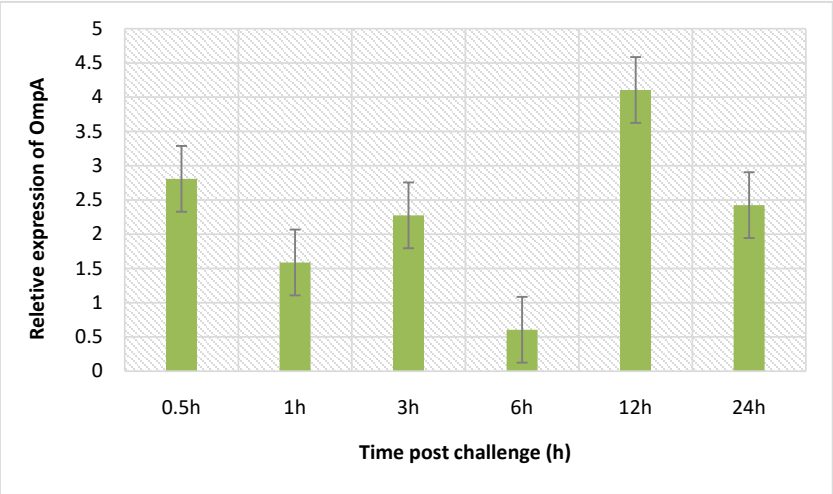

Figure 10. Relative expression of OmpA under *Cinnamomum cassia*.

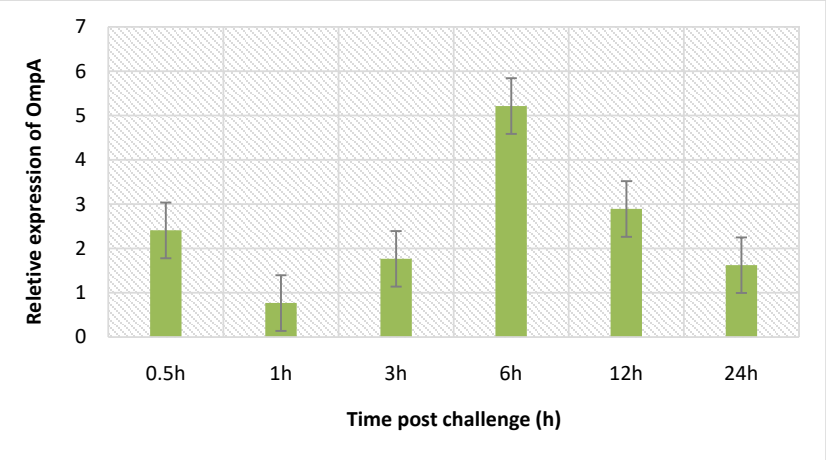

Figure 11. Relative expression of OmpA under *Dolichos lablab L.*

The one-way ANOVA was conducted to compare the mean values of the four groups (*A. membranaceus* (A), *A. sativum* (G), *C. cassia* (C), and *D. lablab L.* (W)). The results show that there is a statistically significant difference between the means of the groups ($F(3, 20) = 10.11385$, $p = 0.00029 < 0.05$). Since the p-value is less than the significance level of 0.05, we reject the null hypothesis, which means there is a significant difference between at least two of the groups. The between-group sum of squares (SS) is 28.97447, and the within-group sum of squares is 19.09888. This indicates that there is more variation between the groups than within the groups. Group A has a mean value of 0.136633 and a variance of 0.023783. Group G has a mean value of 0.221927 and a variance of 0.049898. Group C has a mean value of 2.300905 and a variance of 1.383496. Group W has a mean value of 2.446222 and a variance of 2.362599. From the mean values, we can observe that groups C and W have substantially higher mean values compared to groups A and G. This suggests that groups C and W are experiencing a different effect compared to groups A and G.

In conclusion, the one-way ANOVA analysis indicates that there is a significant difference between the four groups.

4. Discussion

The mechanism for bacteria to protect themselves against essential oils may involve an initial inhibition of OmpA expression by the herbs. OmpA, a protein that helps bacteria survive under stress [10], might then be destroyed by the herbs. In response, bacteria adapt by increasing OmpA expression to overcome the stress. The initial downregulation of OmpA expression by all herbs might result from an increase in extracytoplasmic sigma factor SigmaE and MicA, or by giving a false signal suggesting an overexpression of outer membrane proteins. Another explanation could be that small solutes in the herbs passively diffuse into the bacteria through OmpA, causing a negative internal pressure and damaging the OmpA as they pass through.

Astragalus membranaceus essential oil's ability to continuously suppress OmpA gene expression aligns with its proven strong and sustainable bactericidal effects. *Allium sativum* essential oil's overall OmpA expression being lower than 1 is consistent with its strong bactericidal effect on *E. coli*, although the increase in expression between 6 to 12 hours suggests the beginning of bacterial resistance development. The higher overall expression values for *Cinnamomum cassia* and *Dolichos lablab L.* are also consistent with their moderate inhibitory abilities and persistency.

There were some limitations to this study. Spectrometer measurements varied, and despite numerous trials, potential errors may still exist. The lab environment in our high school may not be as controlled as those in universities or research centers, possibly contributing to discrepancies in results. However, after discovering this inaccuracy, the experiments were carried on in a more constructed lab, which might have improved the preciseness of our data.

Future research could investigate the detailed pathways through which herbs impact *E. coli* biofilm formation and metabolism, offering a more comprehensive understanding of the underlying mechanisms. Identifying specific active molecules in the herbs that contribute to these effects could enable targeted and efficient applications. Moreover, exploring alternative materials or compounds that enhance antibacterial treatment may lead to novel therapeutic strategies against *E. coli* infections.

5. Conclusion

All four herbs—*Astragalus membranaceus*, *Allium sativum*, *Cinnamomum cassia*, and *Dolichos lablab L.* demonstrate inhibitory effects at all tested concentrations. *Astragalus membranaceus* exhibits the strongest performance in terms of antibacterial potency at low concentrations, resistance prevention, and effectiveness in inhibiting Outer membrane protein A (OmpA) expression. *Allium sativum* is also a powerful bactericide, but its efficacy appears to be short-lived, with *E.*

coli more likely to develop resistance due to its ability to restore OmpA expression. *Cinnamomum cassia* has a relatively weaker inhibitory effect on *E. coli*; however, it excels at preventing bacterial resistance. *Dolichos lablab L.* exhibits the weakest performance overall compared to the other herbs. OmpA expression is associated with both antibacterial effects and the development of bacterial resistance.

In a comprehensive summary by Costa *et al.* [14], *Astragalus membranaceus* is reported to have antioxidant, anti-apoptotic, and anti-inflammatory properties in humans. These additional benefits offer further insights into its potential use for treating related conditions. Similarly, *Allium sativum* not only exhibits antibacterial effects but also demonstrates antimicrobial, antioxidant, and antiinflammatory activities [15]. *Cinnamomum cassia* also possesses antiviral, antitumor, and anti-inflammatory properties [16]. In a study by An *et al.* [17], *Dolichos lablab L.* was found to significantly alleviate gastric stress-related mucosal diseases. Overall, these herbs offer a range of health benefits beyond their antibacterial effects, which could contribute to their potential use in treating various conditions.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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