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The Antimicrobial Efficacy of Ascorbic Acid Against Several Strains of *Escherichia coli*

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Abstract

Objective: Ascorbic acid's acknowledged antioxidant and immune-stimulating qualities led to an increase in interest in its antibacterial qualities. Nonetheless, comprehending the impact of ascorbic acid on distinct *Escherichia coli* strains continues to be challenging.

Methods: In this work, four strains of *Escherichia coli*—enterotoxigenic, enteroinvasive, enteropathogenic, and enteroaggregative—were treated with three different doses of ascorbic acid: 5, 10, and 20 mg/ml. *Escherichia coli* strain vitality and proliferation were assessed using spectrophotometer absorbance readings after treatment.

Results: On the treated strains, there was a discernible dose-dependent inhibitory effect. The absorption dropped off significantly from 0.310 ± 0.082 when compared to the positive control (0 mg/ml) to 0.102 ± 0.017 ($P < 0.001$) following the administration of 20 mg/ml of vitamin C. Ascorbic acid at a 5 mg/ml concentration demonstrated the greatest inhibition from 0.310 ± 0.082 to 0.125 ± 0.025 ($P < 0.001$), despite the little incremental changes. Enteroaggregative *Escherichia coli* exhibited the most amount of inhibition, according to our findings.

Conclusion: In the end, the experiment showed that ascorbic acid inhibits *Escherichia coli* growth. According to the studies, at lower doses, ascorbic acid may be able to limit the growth of certain strains of *Escherichia coli*. To find out how much ascorbic acid is best consumed in human situations or to comprehend the underlying mechanisms that contribute to its beneficial benefits, more research is needed.

Keywords: Antimicrobial, ascorbic acid, *Escherichia coli,* antimicrobial inhibition

Introduction

Increased immunity is a known benefit of ascorbic acid. Recent studies have concentrated on its antibacterial properties. The bacterium *Escherichia coli* is frequently found in the stomachs of both humans and animals.[1,2] The intestines are home to several forms of *Escherichia coli*, however some strains have the capacity to cause fatalities as well as gastrointestinal and urinary issues. Because of the rise of bacteria that are resistant to antibiotics, new treatments for *Escherichia coli* are needed.[3,4] Research on the antibacterial effects of ascorbic acid has been greatly stimulated.[5]

Commonly known as ascorbic acid, ascorbic acid is a strong antioxidant that is necessary for optimal immune system performance.[6] Recent investigations have revealed that ascorbic acid can demonstrate antibacterial activities, specifically targeting *Escherichia coli*.[7] As a result, there was a rise in interest in and excitement about the vitamin's possible healing properties.[8] It was discovered that ascorbic acid inhibited the growth of certain *Escherichia coli* strains.[9] Ascorbic acid has a significant potential as an antibacterial agent since, as demonstrated by a study via Hassuna et al.,[10] large doses of the acid decreased the survivability of *Escherichia coli*. Furthermore, ascorbic acid interferes with the integrity of bacterial membranes and inhibits their metabolic processes,[11,12] and lowers viability and proliferation.[13] Ascorbic acid was also shown to increase the effectiveness of antibiotics against strains of *Escherichia coli* that were resistant to them,[14] suggesting that antibiotic resistance may eventually be resolved.[15]

While the antibacterial activity of ascorbic acid against *Escherichia coli* is promising, more research is still needed to determine the exact doses, methods of action, and potential side effects of ascorbic acid. This study investigates the antibacterial characteristics of ascorbic acid and the ideal dosages required to inhibit the growth of several *Escherichia coli* strains.

Methods

Escherichia coli used Strains

i. *Escherichia coli Enterotoxigenic* (ETEC) produces toxic compounds while adhering to the gut lining with hair-like projections called fimbriae. ETEC is a prevalent cause of traveller's diarrhoea and is known to persuade diarrhoea, which is frequently detected in neonates.[16]
ii. *Escherichia coli Enteroinvasive* (EIEC) gains entry into the colon and eliminates the epithelial cells, causing diarrhea that looks a lot like dysentery. An additional common symptom is pyrexia.[17]
iii. *Escherichia coli Enteropathogenic* (EPEC) binds to the intestinal cells by way of the particular attachment protein intimin. Watery stool is a common symptom of EPEC infection, and it can also occasionally show hematochezia. In low-income countries, EPEC is known to have an impact on babies.[18]
iv. *Escherichia coli Enteroaggregative* (EAEC) demonstrates a distinct adhesion design to the epithelium of the gut, aggregating, and producing enteroaggregative toxin. Pediatric patients frequently experience prolonged diarrhea due to the EAEC strain.[19]

Escherichia coli Strains Culturing and Management

Escherichia coli suspended in peptone water was added to freshly produced broths, which were then incubated for two hours. Using a previously reported methodology by Isela et al., L-Ascorbic Acid was added to (3) different batches of

Trypticase Soy Broth (TSB; Himedia, India) to produce final concentrations of 5, 10, and 20 mg/ml.[20] In test tubes holding 1000 µL of the produced solution, 250 µL of the bacterial broth (*Escherichia coli*) were added as part of the experimental method. After that, tubes were incubated aerobically throughout the entire night at 37°C. Three treatment groups were used in the experimental setup: uninoculated TSB enriched in ascorbic acid, uninoculated TSB devoid of ascorbic acid, and bacterial broths in TSB without ascorbic acid (positive control). The absorbance of the infected broths and controls was measured by spectrophotometric analysis using a microplate reader (BIO-RAD Model 680) set to 450 nm.

Statistical Analysis

An liberated samples *t*-test and SPSS software (SPSS 20.0) were charity for a statistical revision. *P* values were considered statistically significant if they were less than 0.005.

Results

With varying ascorbic acid concentrations, the *Enterotoxigenic Escherichia coli* samples' mean absorbance varied. As the concentration of ascorbic acid increased, the absorbance dropped from 0.140 ± 0.012 to 0.107 ± 0.015 ($P < 0.001$), In contrast, the positive controls (Table 1 or Figure 1) had a high absorbance of 0.310 ± 0.082.

The content of ascorbic acid as well as the average absorbance values of interventions *Escherichia coli* were found to be correlated similarly. Comparing the absorbance to the control groups that where positive (Table 2 or Figure 2), statistical analysis showed a substantial decrease with increasing ascorbic acid content, from 0.130 ± 0.02 to 0.104 ± 0.01 ($P < 0.001$).

The findings show that the mean absorbance values for *enteropathogenic Escherichia coli* and the ascorbic acid concentration have a negative connection. Comparing the absorbance to control groups that where the positive (Table 2 or Figure 2), statistical analysis showed a substantial decrease in absorbance with increasing ascorbic acid content, from 0.125 ± 0.025 to 0.106 ± 0.018 ($P < 0.001$).

The findings show that there is a negative relationship between the mean absorbance values for *enteroaggregative Escherichia coli* and the ascorbic acid concentration. Comparing the absorbance to the positive controls, statistical analysis showed a substantial decrease with increasing ascorbic acid concentration, from 0.127 ± 0.024 to 0.102 ± 0.017 ($P < 0.001$) (Table 3 and Figure 3).

The variance in ascorbic acid absorbance at 5, 10, or 20 mg/ml concentrations was compared to the comparable values of ascorbic acid at the groups 0, 5, & 10 mg/ml absorptions using a comparison analysis. There was no statistically significant changes between the absorption ethics of *Escherichia coli* to Ascorbic acid absorptions at the groups 10 & 20 mg/ml (when *P* is 0.315), despite the point of the concentration which where 20 mg/ml of Ascorbic acid demonstrated the strongest repression of *Escherichia coli*. As a result, the findings demonstrated that ascorbic acid's inhibitory effect stops at a absorption of the group 10 mg/ml. However, there were statistically significant changes in *Escherichia coli* absorption ranks ($P < 0.001$) between the variety groups of 0–5 mg/ml & 5–10 mg/ml of ascorbic acid dealings (Table 4 & Figure 4).

Table 1. **Compares the absorption results for *Escherichia coli* which is toxic *(Enterotoxigenic Escherichia coli)* after being treated with ascorbic acid**

Dealing groups	(Mean ± SEM)	*P* value
(Ascorbic acid control)	**(0.089 ± 0.010) d**	**0.003 ****
(Negative control)	**(0.090 ± 0.013) d**	
Positive control (0 mg/ml)	**(0.310 ± 0.082) a**	
Ascorbic acid (5 mg/ml)	**(0.140 ± 0.012) b**	
Ascorbic acid (10 mg/ml)	**(0.111 ± 0.013) c**	
Ascorbic acid (20 mg/ml)	**(0.107 ± 0.015) c**	

The same letters means no differences between them at *P*-value < 0.01.

Table 2. **Compares the absorption results for *Escherichia coli* which is pathogenic *(Enteropathogenic Escherichia coli)* after being treated with ascorbic acid**

Dealing groups	(Mean ± SEM)	*P* value
(Ascorbic acid control)	**(0.089 ± 0.010) d**	**0.014 ****
(Negative control)	**(0.090 ± 0.013) d**	
Positive control (0 mg/ml)	**(0.310 ± 0.082) a**	
Ascorbic acid (5 mg/ml)	**(0.125 ± 0.025) b**	
Ascorbic acid (10 mg/ml)	**(0.109 ± 0.013) c**	
Ascorbic acid (20 mg/ml)	**(0.106 ± 0.018) c**	

The same letters means no differences between them at *P*-value < 0.01.

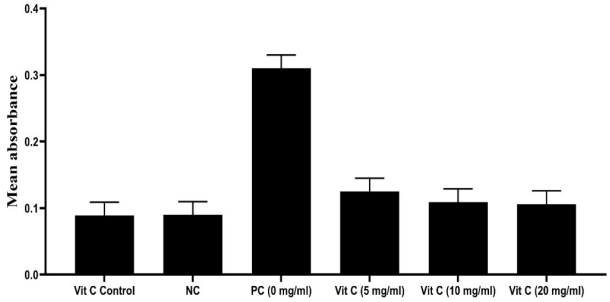

Fig.1 **Compares the absorption results for Escherichia coli which is toxic *(Enterotoxigenic Escherichia coli)* after being treated with ascorbic acid.**

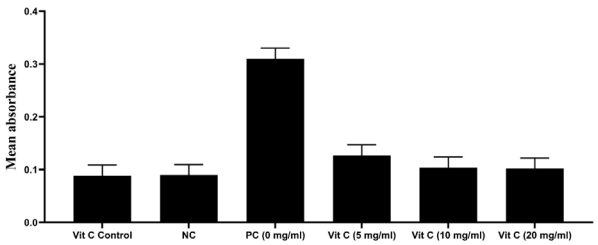

Fig. 2 **Compares the absorption results for Escherichia coli which is pathogenic *(Enteropathogenic Escherichia coli)* after being treated with ascorbic acid.**

Table 3. **Compares the absorption results for Escherichia coli which is aggregative *(Enteroaggregative Escherichia coli)* after being treated with ascorbic acid**

Dealing groups	(Mean ± SEM)	*P*- value
(Ascorbic acid control)	**(0.089 ± 0.010) d**	
(Negative control)	**(0.090 ± 0.013) d**	
Positive control (0 mg/ml)	**(0.310 ± 0.082) a**	**0.007 ****
Ascorbic acid (5 mg/ml)	**(0.127 ± 0.024) b**	
Ascorbic acid (10 mg/ml)	**(0.104 ± 0.013) c**	
Ascorbic acid (20 mg/ml)	**(0.102 ± 0.017) c**	

The same letters means no differences between them at *P*-value < 0.01.

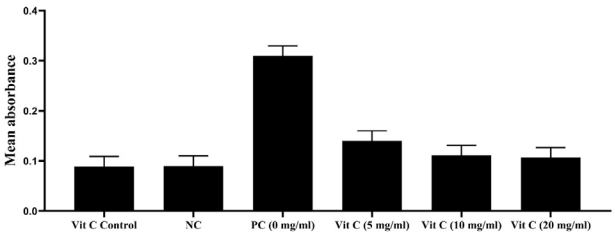

Fig. 3 **Compares the absorption results for Escherichia coli which is aggregative *(Enteroaggregative Escherichia coli)* after being treated with ascorbic acid.**

Discussion

A significant global health problem is the predominance of enteric infections caused by various pathogenic strainings of *Escherichia coli*, such as enterotoxigenic, enteroinvasive, enteropathogenic, and enteroaggregative strains.[21] Researchers have been looking on potential treatments for these infections, like using ascorbic acid.[22] Different strains of *Escherichia coli* cause different kinds of gastrointestinal disorders. ETEC causes diarrhoea through the generation of toxins,[23] but EIEC can penetrate and harm intestinal cells. *Escherichia coli* comes in two varieties: EPEC and EAEC, and they interact with intestinal cells in different ways.[24] Whereas EPEC is recognized to adhere to the gut cells and form "joining and exposing" lacerations, EAEC is identified to produce a biofilms inside digestive region. All strains retains A unique pathogenic process.[25]

A vital nutritional component known for it's antioxidative properties and role in immune system function is ascorbic acid (Ascorbic acid).[26,27] It has been discovered that ascorbic acid possesses antibacterial qualities, such as the capacity to hinder the growth and pathogenicity of *Escherichia coli* strains.[9,28] Ascorbic acid has the potential to reduce the pathogenicity of *Escherichia coli* by disrupting bacterial cell membranes, impeding bacterial adhesion, and inhibiting the generation of toxins.[29,30]

There is still much to learn about the exact mechanism by which ascorbic acid inhibits the growth of *Escherichia coli* strains. However, research studies suggest a number of possible pathways, including the possibility that ascorbic acid could weaken the bacterial cell membrane's structural stability.[31] It has been discovered that giving ascorbic acid to bacteria causes oxidative stress, which damages the cell's membrane and other internal components, among other structural elements.[32] The release of intracellular components could have a negative impact on the survival and functionality of the bacterial cells. When essential cellular operations are interfered with, the bacteria may not be able to maintain its normal physiological functioning. *Escherichia coli* growth can be inhibited by the loss of cellular integrity and impaired structural stability.[31]

Depending on the type of strain and the proposed use, there may be variations in the ascorbic acid concentration that works best against *Escherichia coli* bacteria.[32] Numerous investigations have been carried out to assess the effectiveness of various concentrations. Research has indicated that focuses in the 1–10 mm series may inhibit *Escherichia coli* growing and pathogenicity. Specifically, concentrations higher than 20 mM

Table 4. **The variation in relative absorbance between *Escherichia coli* strains as the concentration of ascorbic acid increases**

Enterotoxigenic Escherichia coli		
Ascorbic acid (mg/ml)	**Mean ± SEM**	***P*-value**
Control (Positive groups) 0	**(0.310 ± 0.082) a**	
(5)	**(0.140 ± 0.012) b**	
(5)	**(0.140 ± 0.012) b**	**0.002 ****
(10)	**(0.111 ± 0.026) c**	
(10)	**(0.111 ± 0.014) c**	
(20)	**(0.107 ± 0.019) c**	
The same letters means no differences between them at *P*-value < 0.01.		
Enteroinvasive Escherichia coli		
Ascorbic acid (mg/ml)	**Mean ± SEM**	***P*-value**
Control (Positive groups) 0	**(0.310 ± 0.082) a**	
(5)	**(0.130 ± 0.02) b**	
(5)	**(0.130 ± 0.02) b**	**0.042 ***
(10)	**(0.107 ± 0.01) c**	
(10)	**(0.107 ± 0.01) c**	
(20)	**(0.104 ± 0.01) c**	
Enteropathogenic Escherichia coli		
Ascorbic acid (mg/ml)	**Mean ± SEM**	***P*-value**
Control (Positive groups) 0	**(0.310 ± 0.082) a**	
(5)	**(0.125 ± 0.025) b**	
(5)	**(0.125 ± 0.025) b**	**0.029 ***
(10)	**(0.109 ± 0.013) c**	
(10)	**(0.109 ± 0.013) c**	
(20)	**(0.106 ± 0.018) c**	
Enteroaggregative Escherichia coli		
Ascorbic acid (mg/ml)	**Mean ± SEM**	***P*-value**
Control (Positive groups) 0	**(0.310 ± 0.082) a**	
(5)	**(0.127 ± 0.024) b**	
(5)	**(0.127 ± 0.024) b**	**0.035***
(10)	**(0.104 ± 0.013) c**	
(10)	**(0.104 ± 0.013) c**	
(20)	**(0.102 ± 0.017) c**	
The same letters means no differences between them at *P*-value < 0.05.		

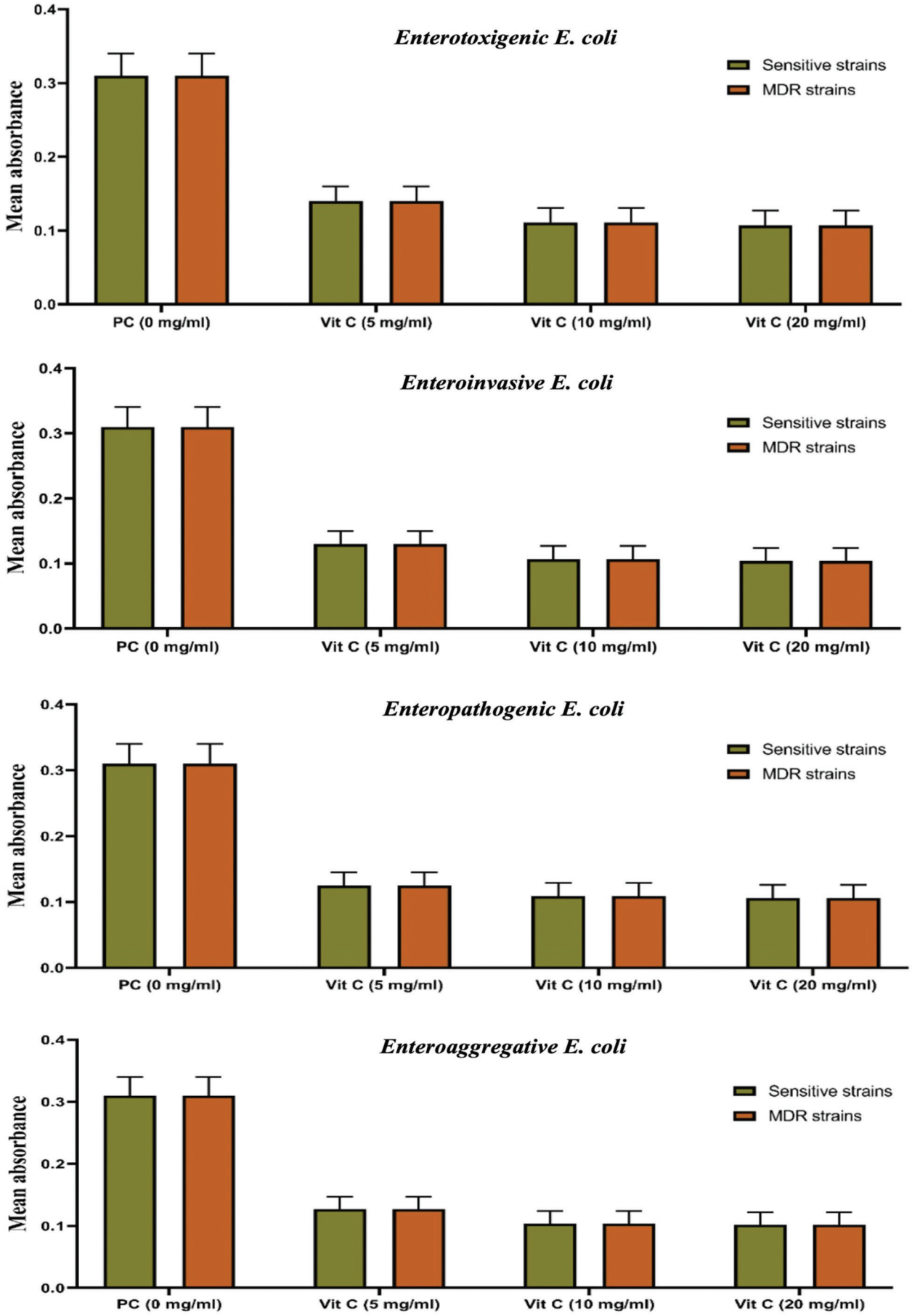

Fig. 4 **The variation in relative absorbance between Escherichia coli strains as the concentration of ascorbic acid increases.**

have the potential to cause cytotoxicity to host-derived healthy cells, while concentrations lower than this threshold might not have significant inhibitory effects.[33]

Due to small size of the sample only 4 different types of *Escherichia coli* strains were included—our findings might not apply to the total population of these strains. The small sample size could restrict how far the results can be applied. The four *Escherichia coli* strains chosen for the study had minimal genetic variety, which could lead to insufficient variation within the species. This issue may therefore limit the data' generalizability to other strains of *Escherichia coli*. In order to guarantee an accurate assessment of the effect of ascorbic acid, appropriate control groups must be included. Without adequate controls, it becomes challenging to determine whether the observed effects are solely due to ascorbic acid or if there are other contributing factors.

The impact of ascorbic acid was assessed quantitatively using the absorbance intensity of *Escherichia coli* cells. Measuring with accuracy and objectivity may help increase the study's repeatability and dependability. Furthermore, the absorption intensity measurement demonstrates a high level of sensitivity, making it possible to identify even minute differences in the samples. Because of this increased sensitivity, it is possible to identify even the smallest differences in how *Escherichia coli* strains react to different Ascorbic acid concentrations. Our findings, which are intriguing, suggest that ascorbic acid has the ability to considerably ($P < 0.001$) affect the survivability of the four strains of *Escherichia coli*, suggesting that it may find application as an antibacterial agent.

Conclusion

As a preventive and therapeutic measure against infections brought on by different strains of *Escherichia coli*, ascorbic acid shows promise. The capacity of ascorbic acid to rupture bacterial cell membranes, obstruct adhesion, and prevent the formation of toxins is thought to be the cause of its inhibitory effects on the development and pathogenicity of *Escherichia coli*. To ascertain the ideal concentration and fully clarify the underlying mechanisms, more research is necessary. But more research is needed to fully understand the potential of ascorbic acid as an additional intervention in the treatment of *Escherichia coli* infections.

Conflict of Interest

None.

References

1. Axe J. Ancient Remedies for Modern Life: from the bestselling author of Keto Diet. Hachette UK; 2021 Feb 4.
2. Lawal OU, Parreira VR, Goodridge L. The biology and the evolutionary dynamics of diarrheagenic *Escherichia coli* pathotypes. In *Escherichia coli* 2022 Feb 3. IntechOpen.
3. Mann R, Mediati DG, Duggin IG, Harry EJ, Bottomley AL. Metabolic adaptations of uropathogenic *Escherichia coli* in the urinary tract. Frontiers in Cellular and Infection Microbiology. 2017 Jun 8;7:241.
4. Wood SJ, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa:* Infections, Animal Modeling, and Therapeutics. Cells. 2023 Jan 3;12(1):199.
5. Ghosh T, Srivastava SK, Gaurav A, Kumar A, Kumar P, Yadav AS, Pathania R, Navani NK. A combination of linalool, ascorbic acid, and copper synergistically triggers reactive oxygen species and DNA damage and inhibits *Salmonella enterica* subsp. *enterica* Serovar Typhi and *Vibrio fluvialis*. Applied and Environmental Microbiology. 2019 Feb 15;85(4):e02487-18.
6. Firouzi S, Pahlavani N, Navashenaq JG, Clayton ZS, Beigmohammadi MT, Malekahmadi M. The effect of Ascorbic acid and Zn supplementation on the immune system and clinical outcomes in COVID-19 patients. Clinical Nutrition Open Science. 2022;44:144–154.
7. Abdussalam-Mohammed W, Abraheem MS, Mezoughi AB, Ettarhouni ZO, Dakhil OO. Novel Compatible Silver Nanoparticles Functionalised by Ascorbic acid and its Derivatives: Characterisation and their Antibacterial Activity against *Escherichia coli* and *Staphylococcus aureus*. Biointerface Research in Applied Chemistry. 2023;13(6):1–13.
8. Corbo MR, Bevilacqua A, Petruzzi L, Casanova FP, Sinigaglia M. Functional beverages: the emerging side of functional foods: commercial trends, research, and health implications. Comprehensive Reviews in Food Science and Food Safety. 2014 Nov;13(6):1192–206.
9. Max BL, Angolile CM, Raymond VG, Mashauri HL. The dawn of repurposing vitamins as potential novel antimicrobial agents: A call for global emergency response amidst AMR crisis. Health Science Reports. 2023 May;6(5):e1276.
10. Hassuna NA, Rabie EM, Mahd WK, Refaie MM, Yousef RK, Abdelraheem WM. Antibacterial effect of ascorbic acid against uropathogenic *Escherichia coli* in vitro and in vivo. BMC Microbiology. 2023 Dec;23(1):1–2.
11. Addorisio SR, Shteynberg R, Dasilva M, Mixon J, Mucciarone K, Vu L, Arsenault K, Briand V, Parker S, Smith S, Vise C. Oxidative Stress Response in Bacteria: A Review. Fine Focus. 2022 Jul 18;8(1):36–46.
12. Poli V, Madduru R, Aparna Y, Kandukuri V, Motireddy SR. Amelioration of cadmium-induced oxidative damage in wistar rats by Ascorbic acid, zinc and N-acetylcysteine. Medical Sciences. 2022 Jan 26;10(1):7.
13. Atila D, Karataş A, Keskin D, Tezcaner A. Pullulan hydrogel-immobilised bacterial cellulose membranes with dual-release of ascorbic acid and E for wound dressing applications. International Journal of Biological Macromolecules. 2022 Oct 1;218:760–74.
14. Doughari JH, Human IS, Bennade S, Ndakidemi PA. Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. Journal of Medicinal Plants Research. 2009;3(11): 839–848.
15. Hamed S, Emara M. Antibacterial and Antivirulence Activities of Acetate, Zinc Oxide Nanoparticles, and Ascorbic acid Against *E. coli* O157: H7 and *P. aeruginosa*. Current Microbiology. 2023 Feb;80(2):57.
16. Chrienová Ž. Carbohydrate recognition of the novel colonisation factor CS30 of enterotoxigenic *E. coli*. 2017.
17. Panarelli NC. Infectious Diseases of the Gastrointestinal Tract. Gastrointestinal and Liver Pathology. Third Edition, Elsevier. 2022:243–98.
18. *Escherichia coli*, Diarrheagenic. CDC Yellow Book 2024. Available at: https:wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/escherichia-coli-diarrheagenic. Accessed on 1st June 2024.
19. Govindarajan DK, Viswalingam N, Meganathan Y, Kandaswamy K. Adherence patterns of *Escherichia coli* in the intestine and its role in pathogenesis. Medicine in Microecology. 2020 Sep 1;5:100025.
20. Isela S, Sergio N, Jose M, Rene H, Claudio C. Ascorbic acid on oral microbial growth and biofilm formation. Pharma Innovation. 2013;2:104–9.
21. Huang TD, Bauraing C, Bogaerts P, Berhin C, Glupczynski Y. Diagnostics of antibiotic resistance. CMI. 2007;13:S109–S608.
22. González de Llano D, Moreno-Arribas MV, Bartolomé B. Cranberry polyphenols and prevention against urinary tract infections: relevant considerations. Molecules. 2020 Aug 1;25(15):3523.
23. Dubreuil JD, Isaacson RE, Schifferli DM. Animal enterotoxigenic *Escherichia coli*. EcoSal Plus. 2016 Sep 8;7(1).
24. Aminian F, Moshirsadri A, Bahrami S, Bahrami S. *Escherichia coli* bacteria causing diarrhea, threatening travelers in the world, a narrative review. International Journal of Travel Medicine and Global Health. 2023 Mar 1;11(1).

25. Rodriguez-Valverde D, Giron JA, Hu Y, Nataro JP, Ruiz-Perez F, Santiago AE. Highly-conserved regulatory activity of the ANR family in the virulence of diarrheagenic bacteria through interaction with master and global regulators. Scientific Reports. 2023 Apr 29;13(1):7024.
26. Jafari D, Esmaeilzadeh A, Mohammadi-Kordkhayli M, Rezaei N. Ascorbic acid and the immune system. Nutrition and Immunity. 2019:81–102.
27. Yin X, Chen K, Cheng H, Chen X, Feng S, Song Y, Liang L. Chemical stability of ascorbic acid integrated into commercial products: A review on bioactivity and delivery technology. Antioxidants. 2022 Jan 13;11(1):153.
28. Zhou F, Wang D, Hu J, Zhang Y, Tan BK, Lin S. Control Measurements of *Escherichia coli* Biofilm: A Review. Foods. 2022 Aug 16;11(16):2469.
29. Akpoka AO, Erifeta G, Akinyeye AJ, Solanke EO, Izevbuwa OE. Comparative Antimicrobial Effects of Ethanolic and Aqueous Extracts of the Stem and Root of *Moringa oleifera* on some Clinical Isolates (*Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Escherichia coli* and *Candida*. Int J Healthc Sci. 2019;7(1):141–7.
30. Mumtaz S, Ali S, Tahir HM, Kazmi SA, Mughal TA, Younas M. Evaluation of antibacterial activity of ascorbic acid against human bacterial pathogens. Brazilian Journal of Biology. 2021 Aug 27;83:e247165.
31. Shivaprasad DP, Taneja NK, Lakra A, Sachdev D. In vitro and in situ abrogation of biofilm formation in *Escherichia coli* by ascorbic acid through ROS generation, disruption of quorum sensing and exopolysaccharide production. Food Chemistry. 2021 Mar 30;341:128171.
32. McMillan HM, Kuehn MJ. The extracellular vesicle generation paradox: a bacterial point of view. The EMBO Journal. 2021 Nov 2;40(21):e108174.
33. Đukić I, Kaličanin N, Senćanski M, Pajović SB, Milićević J, Prljić J, Paessler S, Prodanović R, Glišić S. Inhibition of SARS-CoV-2 Mpro with Ascorbic acid, L-Arginine and a Ascorbic acid/L-Arginine Combination. Frontiers in Bioscience Landmark. 2023;28(1):8.