

## Antimicrobial Effect of Vitamin C on Different Strains of *E. Coli*

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### Article Information

**Received:** Aug 13, 2023

**Accepted:** Sep, 2023

**Published:** Oct 13, 2023

### Keywords

*Antimicrobial effect, Vitamin C, Ascorbic Acid, E. coli, Antimicrobial resistance.*

### ABSTRACT

In recent years, there has been a growing interest in exploring the potential antimicrobial effects of Vitamin C, known for its antioxidant properties. However, an extensive investigation into the impact of Vitamin C on different strains of *Escherichia coli* (*E. coli*) has been lacking. In this study, four distinct strains, including *Enterotoxigenic*, *Enteroinvasive*, *Enteropathogenic*, and *Enteroadherent* *E. coli* were treated with three concentrations of Vitamin C (5, 10, and 20 mg/ml) to assess its antimicrobial properties. Following treatment, absorbance measurements were taken to evaluate the proliferation and viability of the *E. coli* strains. The results revealed a dose-dependent inhibitory effect across all *E. coli* strains. A dose-dependent inhibitory effect was exhibited by all *E. coli* strains with absorbance significantly decreasing from  $0.310 \pm 0.082$  (positive control with 0mg/ml Vitamin C) to a range of  $0.102 \pm 0.017$  to  $0.107 \pm 0.015$  ( $p < 0.001$ ) when treated with 20 mg/ml of Vitamin C. Despite the slight changes in absorbance, Vitamin C at a concentration of 5 mg/ml showed the most significant inhibitory effect from  $0.310 \pm 0.082$  to a range of  $0.125 \pm 0.025$  to  $0.140 \pm 0.012$  ( $p < 0.001$ ), indicating the potential of Vitamin C to inhibit the growth of different *E. coli* strains even at smaller concentrations. However, our results suggest the importance of strain-specific responses when assessing the antimicrobial efficacy of vitamin C. In conclusion, this study provides positive indications of the ability of Vitamin C to disrupt and reduce *E. coli* proliferation and viability. Nevertheless, further investigations are required to determine the optimal concentration in human-relevant setup and comprehensively elucidate the underlying mechanism of action.

## Introduction

### 1. Introduction

Vitamin C is a nutrient known for its immune-boosting and cold-fighting properties and has been the subject of recent research exploring its potential as an antimicrobial agent.<sup>1</sup> *Escherichia coli* (*E. coli*) is a bacterial species that colonises the intestinal tracts of both humans and animals.<sup>2</sup>

Although several strains of *E. coli* reside in the intestines, certain types can result in severe gastrointestinal infections, urinary tract infections, and potentially fatal ailments.<sup>3</sup> The emergence of antibiotic-resistant strains of *E. coli* has intensified the necessity for alternative strategies to address these infections,<sup>4</sup> prompting a surge of interest in investigating the potential of Vitamin C as an antimicrobial agent.<sup>5</sup>

Ascorbic acid, commonly referred to as Vitamin C, is a potent antioxidant essential for the proper functioning of the immune system.<sup>6</sup> Recent research has revealed the capacity of Vitamin C to demonstrate antimicrobial characteristics, specifically against *E. coli*.<sup>7</sup> Thus, this raised interest and elicited optimism for the vitamin's prospective remedial implementations.<sup>8</sup> Vitamin C was found to impede the proliferation of different strains of *E. coli*.<sup>9</sup> According to a study by Hassuna et al.,<sup>10</sup> the viability of *E. coli* was reduced when exposed to high concentrations of Vitamin C, indicating its high potential as an antimicrobial agent. Additionally, Vitamin C can disturb the membrane integrity of bacteria<sup>11</sup> and obstruct their metabolic processes<sup>12</sup>, ultimately blocking their proliferation and viability.<sup>13</sup> The efficacy of antibiotics against antibiotic-resistant *E. coli* strains can be potentially enhanced by Vitamin C.<sup>14</sup> The observed synergistic effect can potentially address the escalating issue of antibiotic resistance, offering a novel approach to conquer these tenacious bacteria.<sup>15</sup>

Although the potential antimicrobial impact of Vitamin C on *E. coli* is encouraging, additional investigations are required to comprehensively understand its mechanisms of action, optimal dosages, and possible adverse reactions. This study aims to explore the antimicrobial properties of Vitamin C on four strains of *E. coli*, presenting a promising avenue for further investigation and potential implications for human health.

## 2. Methods

### 2.1 Used *E. coli* strains

1. *Enterotoxigenic E. coli* (ETEC) adheres to the intestinal lining through hair-like projections known as fimbriae and generates harmful substances. ETEC is known to induce diarrhoea, frequently observed in neonates, and is a common aetiology of travellers' diarrhoea.<sup>16</sup>
2. *Enteroinvasive E. coli* (EIEC) infiltrates and eradicates the epithelial cells of the colon, resulting in diarrhoea that resembles dysentery. Pyrexia is an additional prevalent symptom.<sup>17</sup>
3. *Enteropathogenic E. coli* (EPEC) adheres to intestinal cells through intimin, a specific attachment protein. The symptomatology of EPEC infection is characterised by watery stool,

which may occasionally exhibit hematochezia. EPEC is known to affect infants in low-economic nations.<sup>18</sup>

4. *Enteroaggregative E. coli* (EAEC) exhibits a unique pattern of adherence to the intestinal epithelium, forming aggregates, and is capable of synthesising enteroaggregative toxin. The EAEC strain is a common cause of persistent diarrhoea in paediatric patients.<sup>19</sup>

## 2.2 *E. coli* strains culture and treatment

Incubation of freshly prepared *E. coli* broths suspended in peptone water was carried out for a duration of 2 hours. L-Ascorbic Acid was introduced into three separate batches of Trypticase Soy Broth (TSB; Himedia, India) to achieve final concentrations of 5, 10, and 20 mg/ml, following a previously published protocol by Isela et al (Reference?). The experimental procedure involved the inoculation of 250  $\mu$ L of the bacterial broth (*E. coli*) into test tubes containing 1000  $\mu$ L of the prepared solution. Subsequently, tubes were incubated overnight at 37°C under aerobic conditions. The experimental setup involved three treatment groups, which included bacterial broths in TSB without Vitamin C (positive control), uninoculated TSB devoid of Vitamin C (negative control), and uninoculated TSB enriched Vitamin C. Absorbance measurements of the inoculated broths and controls were conducted using a microplate reader (BIO-RAD Model 680) at a wavelength of 450 nm via spectrophotometric analysis.

## 2.3 Statistical analysis

Independent samples t-test using SPSS software (SPSS 20.0) was used to perform the statistical analysis. A P value < 0.005 was considered as statistically significant.

## 3. Results

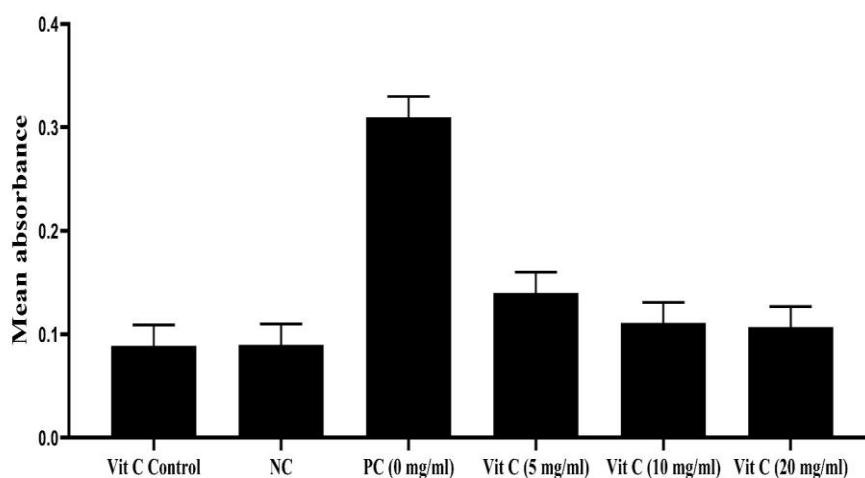
The mean absorbance of the *Enterotoxigenic E. coli* samples varied with different concentrations of vitamin C. Absorbance decreased from 0.140 $\pm$ 0.012 to 0.107 $\pm$ 0.015 (p<0.001) with increasing Vitamin C concentration compared to the high absorbance (0.310 $\pm$ 0.082) in positive controls (Table 1 and Figure 1).

**Table 1. Comparison of the absorbance values of *Enterotoxigenic E. coli* post-treatment with Vitamin C.**

Treatment group	Mean $\pm$ SEM	P value
Vitamin C control	0.089 $\pm$ 0.010	NA
Negative control	0.0899 $\pm$ 0.013	NA
Positive control (0mg/ml)	0.310 $\pm$ 0.082	References
Vitamin C (5 mg/ml)	0.140 $\pm$ 0.012	<0.001*

Vitamin C (10 mg/ml)	0.111±0.013	<0.001*
Vitamin C (20 mg/ml)	0.107±0.015	<0.001*

Results are expressed as Mean ± SEM (n=3). \*Values at p <0.05 are significantly different.



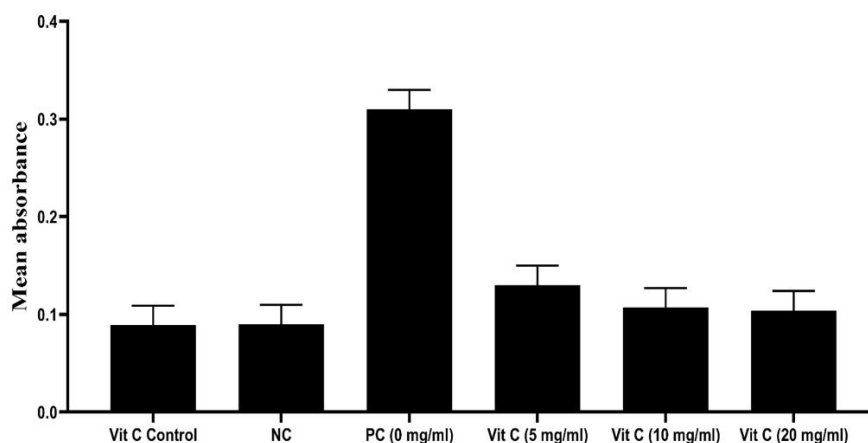
**Figure 1. Comparison of the absorbance values of *Enterotoxigenic E. coli* post-treatment with Vitamin C.**

A similar correlation was observed between the concentration of Vitamin C and the mean absorbance values of *Enteroinvasive E. coli*. Statistical analysis revealed a significant decrease in absorbance from 0.130±0.02 to 0.104±0.01 (p<0.001) with increasing Vitamin C concentration in comparison to the positive controls (Table 2 and Figure 2).

**Table 2. Comparison of the absorbance values of *Enteroinvasive E. coli* post-treatment with Vitamin C.**

Treatment group	Mean±SEM	P value
Vitamin C control	0.089±0.010	NA
Negative control	0.0899±0.013	NA
Positive control (0mg/ml)	0.310±0.082	References
Vitamin C (5 mg/ml)	0.130±0.02	<0.001*
Vitamin C (10 mg/ml)	0.107±0.01	<0.001*
Vitamin C (20 mg/ml)	0.104±0.01	<0.001*

Results are expressed as Mean ± SEM (n=3). \*Values at p <0.05 are significantly different.



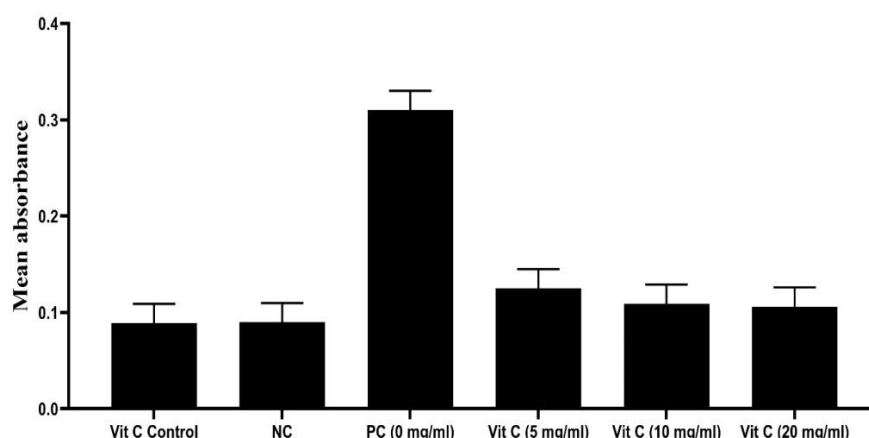
**Figure 2. Comparison of the absorbance values of *Enteroinvasive E. coli* post-treatment with Vitamin C.**

The results indicate a negative correlation between the concentration of Vitamin C and the mean absorbance values for *Enteropathogenic E. coli*. Statistical analysis revealed a significant decrease in absorbance from  $0.125 \pm 0.025$  to  $0.106 \pm 0.018$  ( $p < 0.001$ ) with increasing Vitamin C concentration in comparison to the positive controls (Table 3 and Figure 3).

**Table 3. Comparison of the absorbance values of *Enteropathogenic E. coli* post-treatment with Vitamin C.**

Treatment group	Mean±SEM	P value
Vitamin C control	$0.089 \pm 0.010$	NA
Negative control	$0.0899 \pm 0.013$	NA
Positive control (0mg/ml)	$0.310 \pm 0.082$	References
Vitamin C (5 mg/ml)	$0.125 \pm 0.025$	$<0.001^*$
Vitamin C (10 mg/ml)	$0.109 \pm 0.013$	$<0.001^*$
Vitamin C (20 mg/ml)	$0.106 \pm 0.018$	$<0.001^*$

Results are expressed as Mean  $\pm$  SEM (n=3). \*Values at  $p < 0.05$  are significantly different.



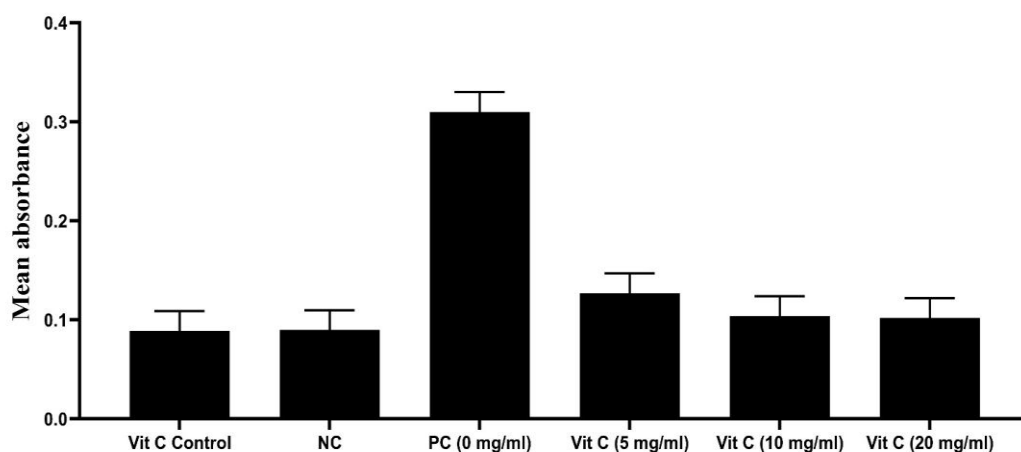
**Figure 3. Comparison of the absorbance values of *Enteropathogenic E. coli* post-treatment with Vitamin C.**

The results indicate a negative correlation between the concentration of Vitamin C and the mean absorbance values for *Enteropathogenic E. coli*. Statistical analysis revealed a significant decrease in absorbance from  $0.127 \pm 0.024$  to  $0.102 \pm 0.017$  ( $p < 0.001$ ) with increasing Vitamin C concentration in comparison to the positive controls (Table 4 and Figure 4).

**Table 4. Comparison of the absorbance values of *Enteropathogenic E. coli* post-treatment with Vitamin C.**

Treatment group	Mean $\pm$ SEM	P value
Vitamin C control	$0.089 \pm 0.010$	NA
Negative control	$0.0899 \pm 0.013$	NA
Positive control (0mg/ml)	$0.310 \pm 0.082$	References
Vitamin C (5 mg/ml)	$0.127 \pm 0.024$	$<0.001^*$
Vitamin C (10 mg/ml)	$0.104 \pm 0.013$	$<0.001^*$
Vitamin C (20 mg/ml)	$0.102 \pm 0.017$	$<0.001^*$

Results are expressed as Mean  $\pm$  SEM (n=3). \*Values at  $p < 0.05$  are significantly different.



**Figure 4. Comparison of the absorbance values of *Enterotoxigenic E. coli* treated with Vitamin C.**

A comparative analysis was conducted to evaluate the variation in the absorbance of Vitamin C at concentrations of 5, 10, and 20 mg/ml with the corresponding measurements of Vitamin C at 0, 5, and 10 mg/ml concentrations. Although the concentration of 20 mg/ml of Vitamin C exhibited the greatest inhibition of *E. coli*, no statistically significant difference was observed in the absorbance values of *E. coli* for Vitamin C concentrations at 10 and 20 mg/ml ( $P = 0.315$ ). Hence, the results showed that the inhibitory effect of Vitamin C does not extend beyond the concentration of 10 mg/ml but statistically significant differences in absorbance levels ( $P < 0.001$ ) of *E. coli* were observed between the ranges of 0 to 5 mg/ml and 5 to 10 mg/ml of Vitamin C treatments (Table 5 and Figure 5).

**Table 5. The relative absorbance difference among *E. Coli* strains with increasing Vitamin C concentrations.**

<i>Enterotoxigenic E. coli</i>		
Vitamin C (mg/ml)	Mean±SEM	P value
Positive control 0	0.310±0.082	NA
5	0.140±0.012	NA
5	0.140±0.012	References
10	0.111±0.026	<0.001*
10	0.111±0.014	<0.001*
20	0.107±0.019	<0.001*
<i>Enteroinvasive E. coli</i>		
Vitamin C (mg/ml)	Mean±SEM	P value
Positive control 0	0.310±0.082	NA
5	0.130±0.02	NA

5	0.130±0.02	References
10	0.107±0.01	<0.001*
10	0.107±0.01	<0.001*
20	0.104±0.01	<0.001*
<b><i>Enteropathogenic E. coli</i></b>		
<b>Vitamin C (mg/ml)</b>	<b>Mean±SEM</b>	<b>P value</b>
<b>Positive control 0</b>	0.310±0.082	NA
5	0.125±0.025	NA
5	0.125±0.025	References
10	0.109±0.013	<0.001*
10	0.109±0.013	<0.001*
20	0.106±0.018	<0.001*
<b><i>Enterocaggregative E. coli</i></b>		
<b>Vitamin C (mg/ml)</b>	<b>Mean±SEM</b>	<b>P value</b>
<b>Positive control 0</b>	0.310±0.082	NA
5	0.127±0.024	NA
5	0.127±0.024	References
10	0.104±0.013	<0.001*
10	0.104±0.013	<0.001*
20	0.102±0.017	<0.001*

Results are expressed as Mean ± SEM (n=3). \*Values at p <0.05 are significantly different.



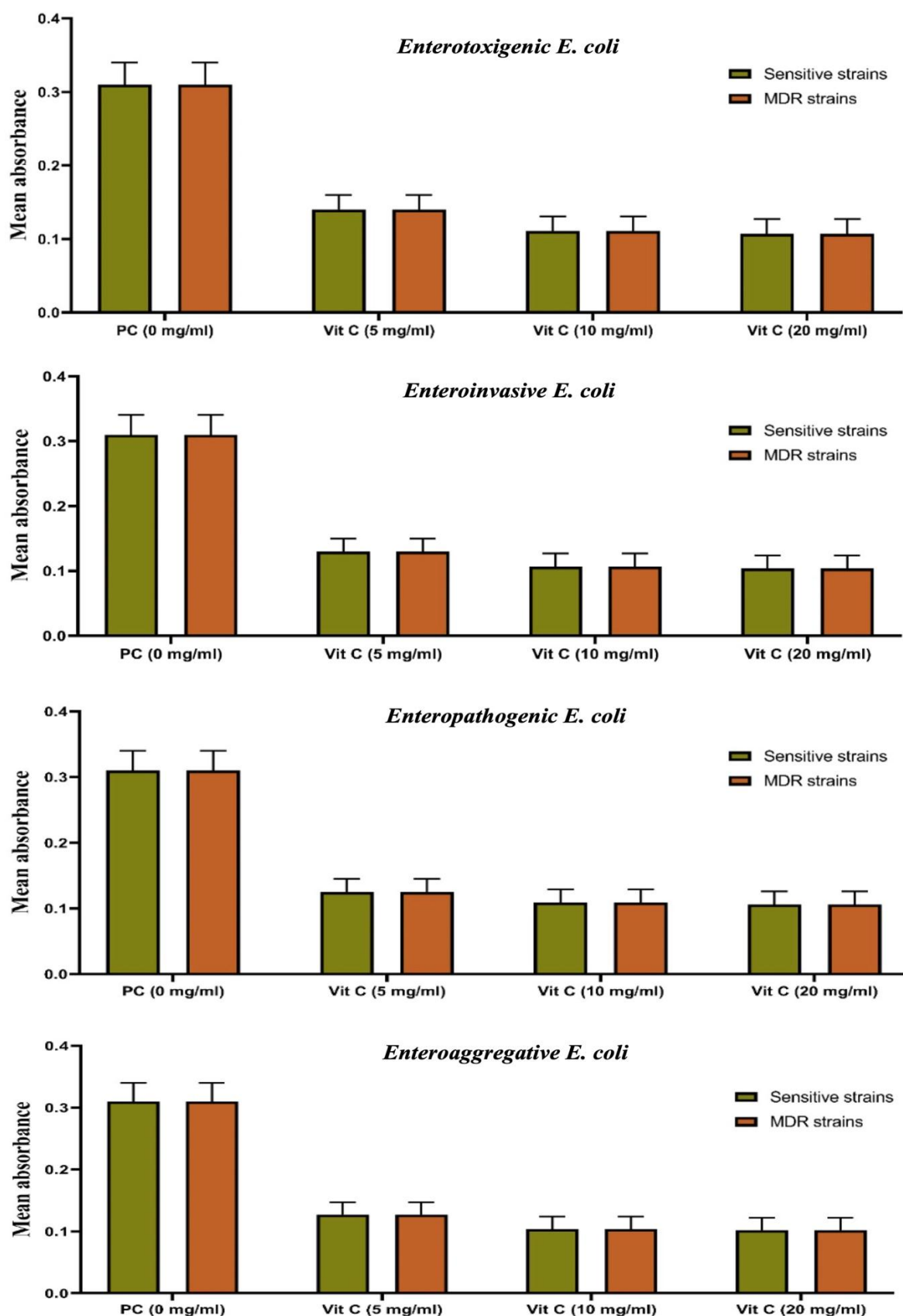


Figure 5. The relative difference in absorbance of the strains of *E. coli* with increasing Vitamin C concentrations.

#### 4. Discussion

The prevalence of enteric infections resulting from diverse pathogenic strains of *Escherichia coli* (*E. coli*), including *Enterotoxigenic*, *Enteroinvasive*, *Enteropathogenic*, and *Enteroadgregative E. coli*, presents a substantial worldwide health concern.<sup>20</sup> Researchers have been investigating prospective interventions to address these infections, such as the utilisation of Vitamin C.<sup>21</sup> There exist discrete strains of *E. coli* responsible for different types of gastrointestinal ailments. The production of toxins by ETEC results in diarrhea,<sup>22</sup> whereas EIEC can invade and inflict damage upon the intestinal cells. EPEC and EAEC are two types of *E. coli* that exhibit distinct modes of interaction with the intestinal cells.<sup>23</sup> EPEC is known to attach to the intestinal cells and create “attaching and effacing” lesions, while EAEC is known to form biofilms within the intestinal tract. Each strain possesses a distinct pathogenic mechanism.<sup>24</sup>

Vitamin C (Ascorbic acid) is a crucial dietary component recognised for its antioxidative characteristics and contribution to the immune system’s functionality.<sup>25, 26</sup> Vitamin C has been found to exhibit antimicrobial properties, which include the ability to obstruct the proliferation and virulence of *E. coli* strains.<sup>27, 28</sup> The potential of vitamin C is rooted in its ability to disrupt bacterial cell membranes,<sup>29</sup> impede bacterial adhesion,<sup>30</sup> and impede toxin production,<sup>31</sup> thereby leading to a reduction in the pathogenicity of *E. coli*.

The complete understanding of the precise mechanism through which Vitamin C obstructs the growth of *E. coli* strains is yet to be explored. Nevertheless, scholarly investigations propose various potential avenues, such as the ability of Vitamin C to compromise the structural stability of the bacterial cell membrane.<sup>31</sup> The administration of Vitamin C was found to elicit oxidative stress in bacterial cells, impairing various structural constituents of the cell, such as the cell membrane and other intracellular structures.<sup>32</sup> The liberation of intracellular components may lead to deleterious consequences on the functional and survival aspects of the bacterial cells. Disruption of crucial cellular processes may impede the ability of the bacterium to uphold its typical physiological functions. The compromised structural stability and loss of cellular integrity can inhibit the growth of *E. coli*.<sup>33</sup>

The concentration of Vitamin C that is most effective against *E. coli* strains may exhibit variability changes with the type of strain and the intended use.<sup>34</sup> Several studies have been conducted to evaluate the efficacy of different concentrations. Studies have demonstrated that concentrations within the 1-10 mM range can impede the growth and virulence of *E. coli*. Notably, concentrations exceeding 20 mM may elicit cytotoxicity in host healthy cells, whereas concentrations below this threshold may not yield substantial inhibitory effects.<sup>35</sup>

Our results may not be generalisable to the entire population of *E. coli* strains owing to the restricted sample size, which included only four types of *E. coli* strains. The diminutive size

of the sample may limit the applicability of the findings. The limited genetic representation of the four *E. coli* strains selected for the study may result in inadequate diversity within the species. Thus, the generalizability of the results to other strains of *E. coli* may be constrained by this factor. To ensure a precise evaluation of the impact of Vitamin C, it is crucial to incorporate suitable control cohorts. Discerning whether the observed effects are exclusively attributable to Vitamin C or other variables becomes difficult without appropriate controls.

The absorbance intensity of *E. coli* strains was utilised as a quantitative metric to assess the impact of vitamin C. Using objective and precise measurements can potentially improve the reliability and reproducibility of the study. In addition, the measurement of absorption intensity exhibits a high degree of sensitivity, enabling the detection of even minor variations in the samples. The heightened sensitivity exhibited by *E. coli* strains enables the detection of minute variations in their response to varying concentrations of Vitamin C. Interestingly, our results showed that Vitamin C could significantly impact ( $p < 0.001$ ) the viability of the four *E. coli* strains, indicating its potential use as an antimicrobial agent.

## 5. Conclusion

Vitamin C exhibits potential as a prophylactic and therapeutic strategy against infections caused by various *Escherichia coli* strains. The inhibitory effects of Vitamin C on the growth and virulence of *E. coli* are attributed to its ability to disrupt bacterial cell membranes, interfere with adhesion, and inhibit toxin production. Additional investigation is required to determine the optimal concentration and comprehensively elucidate the underlying mechanisms. However, the potential of Vitamin C as a supplementary intervention in managing *E. coli* infections is worth further exploration.

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