https://dx.doi.org/10.4314/ijs.v26i2.2

EFFECT OF ANTIOXIDANT PROTECTION AGAINST ULTRAVIOLET RADIATION AND ANTIBIOTIC SUSCEPTIBILITY OF *Escherichia coli*

Agbabiaka, T. O.[1,*], Otuyelu, F. O.[1], Abdulsalam, Z. B.[2] and Mustapha, S.[1]

[1] Department of Microbiology, Faculty of Life Science, University of Ilorin, Nigeria.

[2] Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria.

[*]Corresponding Author's Email: agbabiaka.to@unilorin.edu.ng

(Received: 14th March, 2024; Accepted: 29th April, 2024)

ABSTRACT

Antioxidants possess both biological and physiological properties for preventing damage to cells induced by ultraviolet radiation. The study was conducted to observe the effect of some antioxidants on the survival rate of non-pathogenic and pathogenic strains of *Escherichia coli.* Solutions of Vitamin A, Vitamin C, and Vitamin E were incorporated into sterile Petri dishes containing a fixed amount of the inoculums, the population of the bacterium was determined, and then exposed to ultraviolet light at varying degrees of time. After exposure, Vitamin A reduced cell number at every exposure time, with the highest reduction observed after 20 minutes of exposure time, from 68 cfu/ml to 57 cfu/ml for clinical *E. coli* (E1), while environmental *E. coli* (E2) isolate cell reduction was 41 cfu/ml at 20 minutes observed at concentrations 100 mg/100 ml. For vitamin C at a concentration 100 mg/100 ml, E1 reduced to 8 cfu/ml at 20 minutes. At 100mg/100ml, Vitamin E reduced E2 colonies to 11 cfu/ml at 20 minutes. The antibiotic susceptibility of the isolates showed that E1 showed resistance to 62.5% of the antibiotics tested against, while E2 was resistant to 50% of the antibiotics. This study revealed that at higher antioxidant concentrations, bacterial cells tend to be protected against the effects of UV radiation at a shorter exposure time.

Keywords: antioxidant, ultraviolet, antibiotics, *E. coli,* vitamins

INTRODUCTION

Ultraviolet (UV) light is the portion of the electromagnetic spectrum between visible light and x-rays, with a wavelength of 100 to 400 nm. Ultraviolet radiation could be obtained from the sun, lasers, tanning beds, and medical instruments like dental polymerizing equipment (Kodoth & Jones, 2015). Ultraviolet photons fall between the wavelengths of visible light and gamma radiation.

Bacteria have a single circular chromosome, with occasional plasmids, instead of multiple chromosomes with a fast replication rate (Kuzminov, 2016). Extended exposure to UV radiation can alter the genetic material of bacterial cells, leading to unfavourable mutations and even cell death. The shorter the ultraviolet wavelength, the greater the damage to microorganisms; hence, cell death could be due to the fusion of thymine bases that are located next to each other (Nukala *et al*., 2018).

Nukala *et al.* (2018) reported that ionizing radiation-induced reactive oxygen species (ROS) and reactive nitrogen species (RNS) damage DNA, proteins, and lipids as well as activate intracellular signaling pathways and stimulate cell death in bacteria. Oxidative stress occurs when the generation of free radicals or reactive oxygen species (ROS) exceeds the antioxidant capacity of a biological system, including prokaryotes.

Pullar *et al.* (2018) observed that antioxidants are free-radical scavengers that provide protection to living organisms from damage caused by reactive oxygen species (ROS) due to mutational effects by inducing thymine dimers. Although almost all microorganisms possess antioxidant defense and repair systems but these systems are insufficient to cope with the entire damage. Ascorbic acid is the primary water-soluble antioxidant in plasma and a major non-enzymatic antioxidant in tissues; it can protect many important biomolecules from oxidation as well as regenerate specific molecules, such as vitamin E (VitE) and tetrahydrobiopterin.

Vit E, a fat-soluble antioxidant, stops the production of reactive oxygen species (ROS) formed due to oxidation. VitE is also involved in cell signaling, gene expression regulation, and other physiological and metabolic functions in both the eukaryote and prokaryote cell, respectively (Zingg, 2019). Vitamin C (VitC) is considered the most important water-soluble antioxidant in extracellular fluids, capable of neutralizing reactive oxygen species (ROS) in the aqueous phase before lipid peroxidation is initiated especially cells whose cell walls are rich in lipids, Gram negative bacteria. VitE, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane,

where it protects membrane fatty acids from lipid peroxidation and has demonstrated some protective mechanisms against microorganisms (Nukala *et al.,* 2018). In this study, the effect of some antioxidants on the survival rate of environmental and clinical strains of *Escherichia coli* was examined and compared to the named antibiotics.

MATERIALS AND METHOD

Samples and antioxidants collection

A clinical sample was collected from the University Clinic (site A) and faecally contaminated soil sample was collected from the backyard of the Faculty of Agriculture Laboratory (site B), University of Ilorin, Nigeria. The following antioxidants (Vitamins A, C, and E) were purchased from a pharmaceutical retail outlet in Ilorin, Nigeria.

Bacteriological analysis of *Escherichia coli* isolates

The faecally contaminated soil sample was grown on Eosine Methylene Blue agar and observed for the presence of *E. coli* using the standard microbiological method of isolation as described by Fawole & Oso (2007), purified, and stocked on an agar slant.

Preparation of antioxidants

Antioxidants (based on the recommended daily intake) in the form of VitC, VitA, VitE and a combination of VitC and VitE were prepared as described by the Institute of Medicine (2006).

Determination of inoculum size

From a positive plate of Eosine Methylene Blue agar, distinct colonies of the test microorganism were picked and streaked into liquid nutrient broth, incubated at 37 °C for 48 hours. After inoculation, the broth was centrifuged at 4000 rpm for 20 minutes to separate the supernatant (broth) from the pellet (organism). The pellet was further washed using a phosphate buffer solution until a pure organism was derived. The organism was standardized using 0.5 McFarland standards ($1.5x10^8$ cells/ml).

Antioxidants activity assay and exposure to ultraviolet light

One ml of the antioxidant at different concentrations was pipetted into sterile Petri dishes, 1 ml of standardized test isolate was serially diluted up to 10^4 and added into the Petri dish using the pour plate method as described by Fawole and Osho (2007), sterile nutrient agar was aseptically poured into each of the Petri dishes. Two different controls were adopted; one without antioxidants with no exposure to ultraviolet light and one containing antioxidants but not exposed to ultraviolet radiation. To test the effects of the ultra violet light, the variable Petri dishes were placed exactly 6 inches beneath the ultraviolet lamp within a fume hood (Kodoth and Jones, 2015) at the standard 254 nm wavelength. All plates were labelled accordingly based on antioxidant concentration and the period of exposure (0, 5, 10 and 20 minutes). After exposure, the experimental plates and the control were incubated at 37 °C for 48 hours, after which the plates were observed for growth.

Biochemical tests for identification of *E. coli* isolates

The following biochemical tests carried out on the *E. coli* isolates included Indole, Methyl red, Voges Proskauer, and Citrate test (IMViC). Gram staining was carried out according to the method described by Fawole and Oso (2007).

Antibiotic susceptibility test

Antibiotic susceptibility of the test organisms was performed using the agar disc diffusion method, overnight broth culture of the test organism was standardized using 0.5 MacFarland and the standardized organism was swabbed entirely on the surface of already prepared Muller Hinton agar plates. The antibiotic discs (Ceftazidime CAZ 30 µg; Cefuroxime CRX 3 0µg; Gentamicin GEN 10µg; Ciprofloxacin 5 µg and Nitrofurantoin NIT 300 µg) of Rapid Labs (CM-128NR100), U.K. were placed on the agar surface. The plates were then incubated at 37 °C for 18 to 24 hours, and the zone of inhibition was recorded accordingly (Liu *et al.*, 2016).

RESULTS

Escherichia coli isolation

Purified clinical and environmental isolates (E1 and E2) were further used for other analyses in this study.

Characterization of the bacterial isolates
The bacterial isolates were characterized based on Gram's staining, morphological and biochemical test as shown in Table 1 below:

Table 1: Biochemical test conducted on the test microorganism

Biochemical test	Gram's staining	Indole	Methyl Red	Voges-Proskauer	Citrate
E1	-	+	+	-	-
E2	-	+	+	-	-

KEY: + = Positive, **-** = Negative

Antioxidants activity assay
The results obtained from the activity of VitA on the isolates E1 and E2 at concentration of 40 mg/100 ml and 100 mg/ml are presented in Figures 1 and 2. The total colony forming units (CFUs) recorded on E1 at concentration 40 mg/100ml were 126 cfu/ml at 0 minute, 93 cfu/ml at 5 minutes, 89 cfu/ml at 10 minutes and 68 cfu/ml at 20 minutes, respectively, while at concentration 100 mg were 104 cfu/ml at 0 minute, 82 cfu/ml at 5minutes, 76 cfu/ml at 10 minutes and 57 cfu/ml at 20 minutes, respectively. For E2, at 40 mg/100 ml colonies at different exposure time were 97 cfu/ml at 0 min, 88 cfu/ml at 5 min, 81 cfu/ml at 10 minutes and 64 cfu/ml at 20 minutes, respectively while at 100 mg/100 ml, were 117 cfu/ml at 0 minutes, 95 cfu/ml at 5 minutes, 64 cfu/ml at 10 minutes and 41 cfu/ml at 20 minutes, respectively.

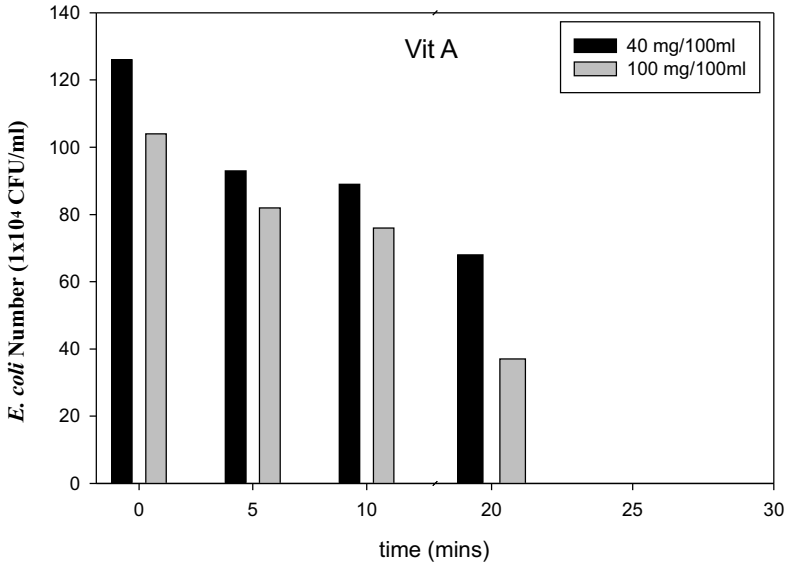

Figure 1: Bacterial count of E1 with VitA after exposure to ultra violet radiation**.**

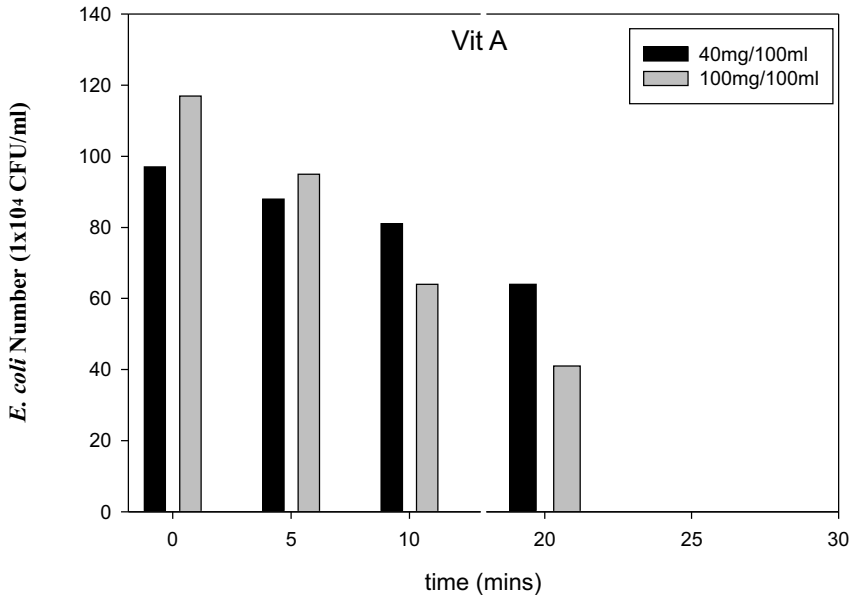

Figure 2: Bacterial count of E2 with VitA with exposure to ultraviolet radiation**.**

Ascorbic acid (VitC) at concentration of 40 mg/100 ml and 100 mg/100 ml were used on the test isolates at different exposure time to UV radiation. For E1, VitC concentration of 40 mg/100 ml showed 61 cfu/ml at 0 minute, 72 cfu/ml at 5 minutes, 47 cfu/ml at 10 minutes and 8 cfu/ml at 20 minutes while at concentration 100 mg/100 ml colonies obtained were 76 cfu/ml at 0 minute, 91 cfu/ml at 5 minutes, 107 cfu/ml at 10 minutes and 84 cfu/ml at 20 minutes. When concentrations 40 mg/100 ml and 100 mg/100 ml was tested on E2, colonies obtained were 106 cfu/ml at 0 minute, 92 cfu/ml at 5 minutes, 76 cfu/ml at 10 minutes, and 11 cfu/ml at 20 minutes, while 118 cfu/ml at 0 minutes, 134 cfu/ml at 5 minutes, 142 cfu/ml at 10 minutes and 127 cfu/ml at 20 minutes were obtained, respectively (Figures 3 and 4).

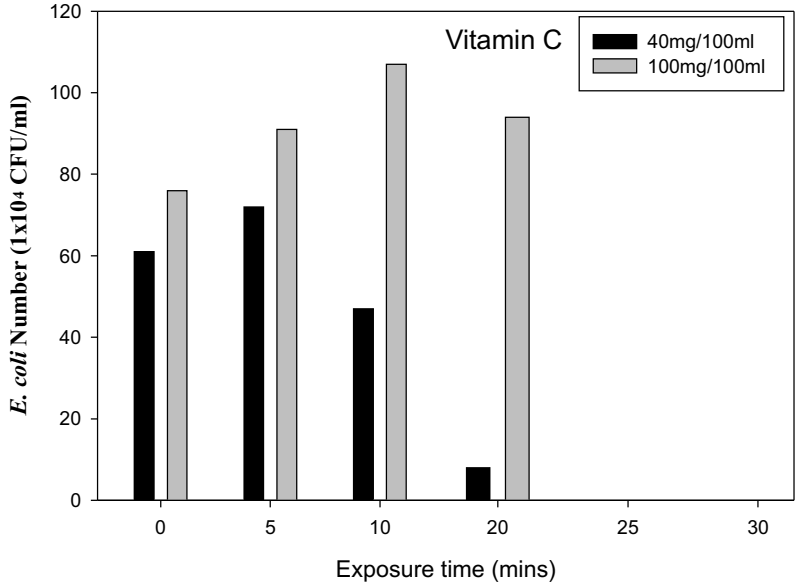

Figure 3: Colony count of E1 incorporated with VitC with exposure to ultra violet radiation

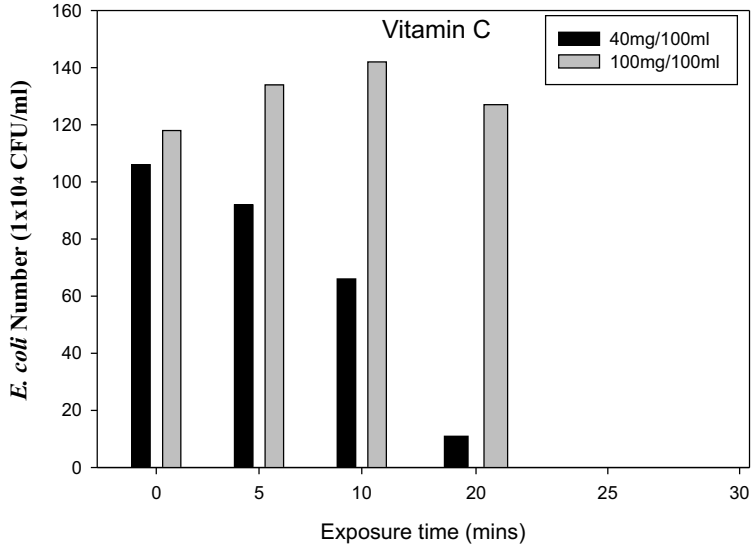

Figure 4: Colony count E2 incorporated with VitC with exposure to ultra violet radiation

The effects of VitE at concentration of 40 mg/100 ml and 100 mg/100 ml on E1 and E2 were presented in Figures 5 and 6. For E1, colonies observed at 40 mg were 86 cfu/ml at 0 minute, 61 cfu/ml at 5 minutes, 42 cfu/ml at 10 minutes and 19 cfu/ml at 20 minutes, while at concentration 100 mg/100 ml colonies observed were 27 cfu/ml at 0 min, 15 cfu/ml at 5 minutes, 12 cfu/ml at 10 minutes and 10 cfu/ml at 20. Consequently, E2 at 40 mg/100 ml showed 51 cfu/ml at 0 minute, 36 cfu/ml at 5 minutes, 21 cfu/ml at 10 minutes and 3 cfu/ml at 20 minutes as against 100 mg/100 ml which showed 69 cfu/ml at 0 minute, 87 cfu/ml at 5 minutes, 72 cfu/ml at 10 minutes and 33 cfu/ml at 20 minutes.

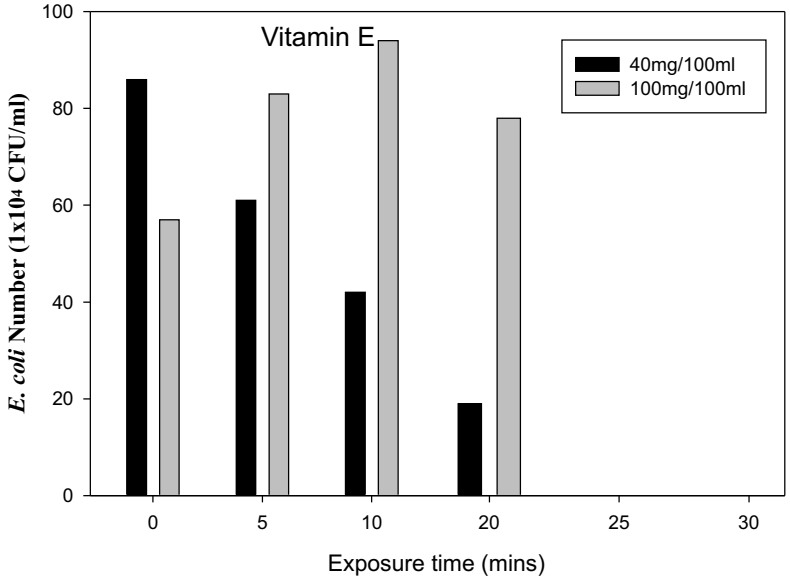

Figure 5: Colony count of E1 containing VitE and exposed to ultra violet radiation

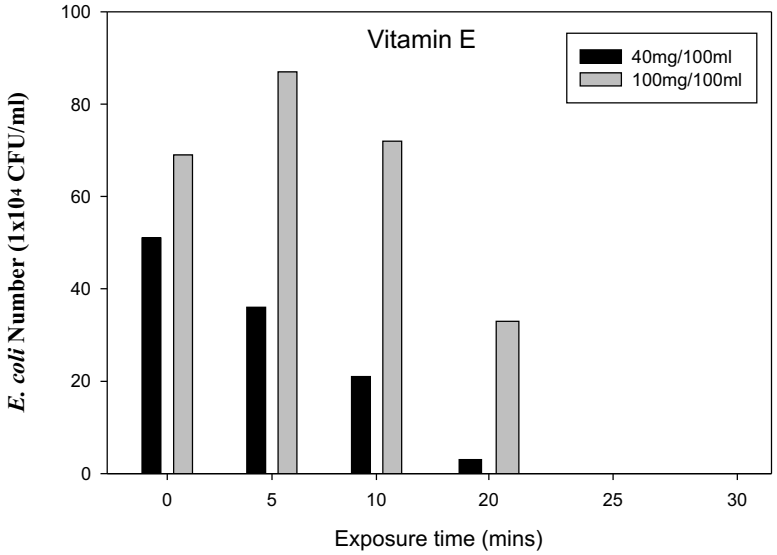

Figure 6: Colony count of E2 obtained from faecally contaminated soil with VitE and exposed to ultra violet radiation**.**

3.4 Activity of VitC and VitE on the isolates

The results of the combination of VitC and VitE on test isolates at 40 mg/100 ml were 84 cfu/ml at 0 minute, 71 cfu/ml at 5 minutes, 45 cfu/ml at 10 minutes and 26 cfu/ml at 20 minutes for E1 while at 100 mg/100 ml were 113 cfu/ml at 0 minute, 92 cfu/ml at 5 minutes, 108 cfu/ml at 10 minutes and 101 cfu/ml at 20 minutes for E1. For E2, at 40 mg/100 ml, 68 cfu/ml at 0 minute, 49 cfu/ml at 5 minutes, 41 cfu/ml at 10 minutes and 13 cfu/ml at 20 minutes while for 100 mg/100 ml, 74 cfu/ml at 0 minutes, 82 cfu/ml at 5 minutes, 99 cfu/ml at 10 minutes and 61 cfu/ml at 20 minutes.

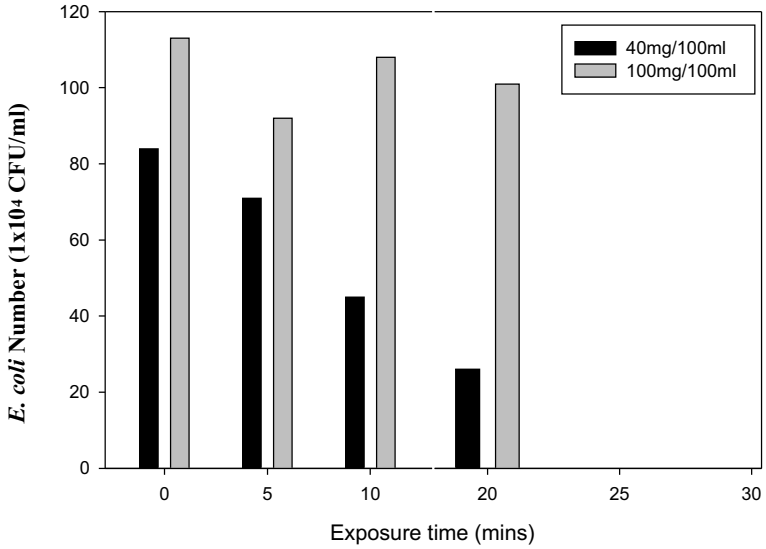

Figure 7: colony count of E1 containing VitC and VitE exposed to ultra violet radiation

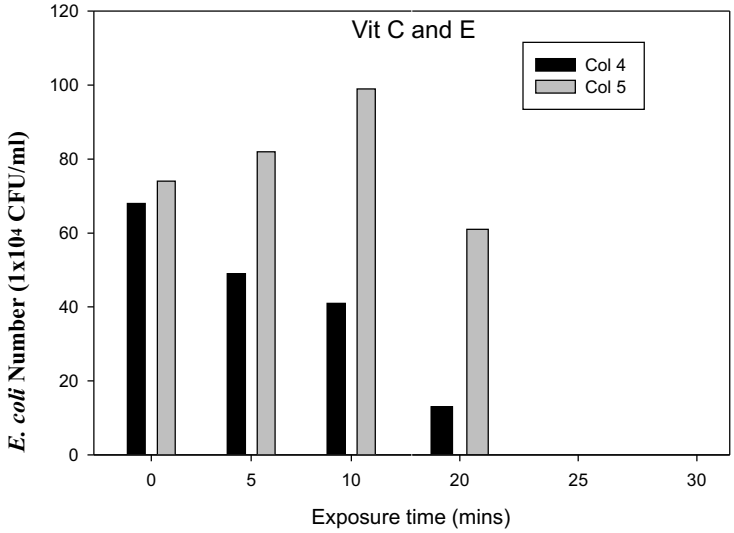

Figure 8: Colony count of E2 containing VitC and VitE exposed to ultraviolet radiation

Antibiotic susceptibility test

The result showed that the clinical isolate (E1) was resistant to 62.5% of the antibiotics tested against while the environmental isolate (E2) was resistant to 50% of the antibiotics (Table 2). For E1, sensitivity was observed against Ciprofloxacin, Ofloxacin and Nitrofurantoin with Ciprofloxacin showing the highest zone of inhibition while E2 was sensitive to Ceftraxidime, Ciprofloxacin, Ofloxacin and Nitrofurantoin with Ciprofloxacin also showing the highest activity of 31.0 ± 3.0 mm. Both isolates showed resistance to Augmentin and Ampicillin at 0.0± 0.0 mm.

Table 2: Antibiotic Susceptibility profile of the two isolates

Isolates	Zone of inhibition (mm)							
	CAZ	**AMP**	**CRX**	**GEN**	**CPR**	**OFL**	**AUG**	**NIT**
E1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	14.6 ± 0.3	29.0 ± 0.0	24.6 ± 0.3	0.0 ± 0.0	19 ± 2.0
E2	26.3 ± 2.9	0.0 ± 0.0	19.3 ± 2.9	14.8 ± 3.7	31 ± 3.0	27.3 ± 2.6	0.0 ± 0.0	21.6 ± 0.8
R	...	⩽13	⩽14	⩽12	⩽15	⩽12	⩽13	⩽14
I	18-20	14-16	15-22	13-14	16-20	13-15	14-17	15-16
S	...	⩾17	⩾23	⩾15	⩾21	⩾16	⩾18	⩾17

KEY

E1- Clinical isolate; E2- Environmental isolate;
CAZ- Ceftraxidime (30 µg); CRX- Cefuroxime (30 µg); CIP- Ciprofloxacin (5 µg); AUG- Augmentin (Amoxycillin Clavulanate (30 µg); AMP- Ampicillin (30 µg); GEN- Gentamicin (10 µg); OFL- Ofloxacin (5 µg); NIT- Nitrofurantoin (300 µg).
R- Resistance; I- Intermediate; S- Sensitive

DISCUSSION

The antioxidant activities of some selected vitamins have been linked to the presence of biologically active compounds that protect cells against the mutational effects of ultraviolet radiation. In this research, the effects of ultraviolet radiation on *Escherichia coli* incorporated with different antioxidants at varying concentrations were investigated. The total number of colony forming units (CFUs) was compared after exposure to ultraviolet radiation at 0, 5, 10, and 20 minutes, respectively. Two control plates were adopted for this study: (plate containing a fixed number of inoculums,

antioxidants but not exposed to ultraviolet, and a plate containing a standardized number of inoculums only. The adoption of two control plates was to determine if the microorganism has the potential to utilize the antioxidants as a growth factor under certain conditions. Consequently, the adoption of ultraviolet radiation with a spectral line of 253 nm for this research was in agreement with that of Singh *et al.* (2013), largely due to its ability to cause substantial biological damage by inducing thymine dimers and consequently causing mutations in bacteria cells. VitC at a concentration of 100 mg/100 ml showed an increase in population of the bacterium with 84 cfu/ml against 8 cfu/ml obtained at 40 mg/100 ml after 20 minutes exposure for E1 while for E2, 100 mg/100 ml showed increase in population of the bacterium with 127 cfu/ml against 11 cfu/ml obtained at 40 mg/100ml after 20 minutes of exposure. The CFUs obtained from the control plate clearly suggested the antioxidant capability of VitC, with a total colony of 72 cfu/ml after 48 hours of incubation. In a study conducted by Verghese *et al.* (2017), where he reported the maximum inhibitory effect of VitC at a concentration of 20 mg/ml on *Escherichia coli*. This also corresponds to the findings of Zhang *et al.* (1997), Biswas *et al.* (2013), Isela *et al.* (2013), and Vilcheze *et al.* (2013), where they reported similar inhibitory effects of VitC on *Staphylococcus aureus*, *Helicobacter pylori*, *Clostridium jejuni,* and *Mycobacterium tuberculosis,* and on fungi, such as *Candida albicans*, *Aspergillus niger,* and *A. flavus*. In contrast to their findings, the concentration of VitC, choice of experimental methods, growth conditions, and strains of microorganisms used could possibly be the reasons for the difference in results obtained. VitC possesses strong antioxidant protective properties, capable of repairing damaged cells induced by ultraviolet radiation, and as demonstrated in research conducted by Biswas *et al.* (2013) and Yin *et al.* (2015). The control plates (plates containing VitC but not exposed to ultraviolet radiation) used in this research exhibited CFUs of 164 cfu/ml, which possibly explained the antioxidant properties of VitC and possibly served as a growth factor for the microorganism under certain conditions.

Concentrations of VitA, (40 mg and 100 mg/100 ml), VitC (40 mg/100 ml), and VitE (40 mg/100 ml) did not protect the bacterial cells when subjected to the mutational effect of ultraviolet radiation, and this could be due to biofilm formation by the microorganism during adverse conditions, as supported by Amar *et al.* (2021) and Isela *et al.* (2013).

VitA has been reported to possess some antioxidant capability, while some reported works claim its pro-antioxidant nature (Nukala, 2018). The two concentrations adopted did not show any significant increase, which might have indicated their contribution to the survival of more *Escherichia coli* colonies.

Pleeging *et al.* (2020) reported that a combination of VitC and VitE demonstrated a protective effect on *Pseudomonas* sp. with considerable survival of the test microorganism. This is very much possible due to the mechanism and combination properties of the two antioxidants. Based on the results obtained after subjecting the two isolates to different antioxidant treatments and exposure to ultraviolet radiation, there is some variability as the isolate obtained from the dumpsite of the University clinic (E1) showed more resistance to the antioxidants used in this study, and this could be due to its pathogenic potentials (Verghese *et al.*, 2017). Maximum reduction in the survival of the isolates was recorded at 20 minutes of exposure time to ultraviolet radiation, as this clearly validates the bactericidal activity at longer exposure time, which conforms to the findings of Kodoth & Jones (2015).

CONCLUSION

The biological protective properties of antioxidants, especially on damaged cells induced by ultraviolet light restore cells to their normal physiological state. While many bacteria possess antioxidant defense mechanisms to protect them against a variety of environmental stresses such as radiation, this mechanism is not sufficient and can be overwhelmed during extreme conditions. In this study, VitC and VitE with the test microorganisms, after exposure to ultraviolet light exhibited an increase in the CFUs obtained as against lower concentrations which clearly indicated that higher concentrations of the two antioxidants had protective ability on E1 and E2.

Although, longer exposure time of the isolates to ultraviolet light resulted in a reduction in cell number.

ACKNOWLEDGMENTS

The Laboratory staff of Department of Microbiology is hereby acknowledged in making some materials available and useful suggestions.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

There is no conflict of interest in any form in this article by any of the authors.

AUTHORS' CONTRIBUTIONS

The first author designed and supervised the project as well as edited the write-up. Samples were collected by second, third and fourth authors. All the authors were involved in the methods used in the study. Proof-reading and additional editing were done by the first and second authors.

REFERENCES

Biswas, S., Thomas, N., Mandal, A., Mullick, A., Chandra, D., Mukherjee, S. and Mitra A. 2013. In vitro analysis of antibacterial activity of Vitamin C alone and in combination with antibiotics on Gram-positive rod isolated from soil of a dumping site of Kolkata. *International Journal of Pharmaceutical and Biological Science,* 3: 101-110.

Fawole, M. O. and Oso, B. A. 2007. Characterization of bacteria: Laboratory Manual of Microbiology. 4th Edition, Spectrum Books Ltd., Ibadan. pp. 15-25.

Institute of Medicine 2006. Dietary reference intakes: the essential guide to nutrient requirements. Eds: Otten, Hellwig and Meyers. National Academies Press, Washington, DC. http://www.nap.edu/catalog/11537.html (accessed 22 November, 2022)

Isela, S., Sergio, N., Jose, M. Rene, H. and Claudio, C. 2013. Ascorbic acid on oral microbial growth and biofilm formation. *Pharma Innovation,* 2: 104-109. https://www.proquest.com/scholarly-journals/ascorbic-acid-on-oral-microbial-growth-biofilm/docview/1924697429/se-2

Kodoth, V. and Jones, M. 2015. The effects of ultraviolet light on *Escherichia coli. Journal of Emerging Investigators,* 102(3): 23-28.

Kuzminov, A. 2016. Chromosomal replication complexity: a novel DNA metrics and genome instability factor. *PLoS Genet.,* 12(10): e1006229. doi 10.1371/journal.pgen.1006229

Liu, H., Taylor, T. H., Pettus, K., Johnson, S., Papp, J. R. and Trees, D. 2016. Comparing the disk-diffusion and agar dilution tests for *Neisseria gonorrhoeae* antimicrobial susceptibility testing. *Antimicrobial Resistance & Infection Control,* 5(1): 1-6. doi 10.1186/s13756-016-0148-x

Nukala, U., Thakkar, S., Krager, K. J., Breen, P. J., Compadre, C. M. and Aykin-Burns, N. 2018. Antioxidant tocols as radiation countermeasures (Challenges to be addressed to use tocols as radiation countermeasures in humans). *Antioxidants,* 7(2): 33. doi: 10.3390/antiox7020033

Pleeging, C. C., Coenye, T., Mossialos, D., De Rooster, H., Chrysostomou, D., Wagener, F. A. and Cremers, N. A. 2020. Synergistic antimicrobial activity of supplemented medical-grade honey against *Pseudomonas aeruginosa* biofilm formation and eradication. *Antibiotics,* 9(12): 866. doi: 10.3390/antibiotics9120866

Pullar, J. M., Bayer, S. and Carr, A. C. 2018. Appropriate handling, processing and analysis of blood samples is essential to avoid oxidation of vitamin C to dehydroascorbic acid. *Antioxidants,* 7(2): 29. doi: 10.3390/antiox7020029

Singh, V., Sivaramaiah, G., Rao, J. L. and Kim, S. H. 2013. Luminescence and electron paramagnetic resonance investigation on ultraviolet emitting Gd doped $MgAl_2O_4$ phosphors. *Journal of Luminescence,* 143: 162-16.
doi: 10.1016/j.jlumin.2013.03.054

Verghese, R. J., Mathew, S. K. and David, A. 2017. Antimicrobial activity of Vitamin C demonstrated on uropathogenic *Escherichia coli* and *Klebsiella pneumoniae. Journal of Current Research in Scientific Medicine,* 3(2): 88.
doi: 10.4103/jcrsm.jcrsm_35_17

Vilcheze, C., Hartman, T., Weinrick, B. and Jacobs, J. 2013. *Mycobacterium tuberculosis* is extraordinarily sensitive to killing by a Vitamin C-induced Fenton reaction. *National Communication*, 4: 1881.
doi: 10.1038/ncomms2898

Yin, F., Zhu, Y., Koutchma, T. and Gong, J. 2015. Inactivation and potential reactivation of pathogenic *Escherichia coli* O157: H7 in apple juice following ultraviolet light exposure at three monochromatic wavelengths. *Food Microbiology,* 46: 329-335.
doi 10.1016/j.fm.2014.08.015

Zhang, H. M., Wakisaka, N., Maeda, O. and Yamamoto, T. 1997. Vitamin C inhibits the growth of a bacterial risk factor for gastrsic carcinoma: *Helicobacter pylori. Cancer*, 80:1897-1903.
doi: 10.1002/(SICI)10970-142(19971115)80:10<1897::AID-CNCR4>3.0.CO;2-L

Zingg, J. M. 2019. Vitamin E: regulatory role on signal transduction. *IUBMB life,* 71(4): 456-478.
doi 10.1146/annurev-nutr-071714-034347.