Available online at www.jpsscientificpublications.com

Life Science Archives (LSA)

ISSN: 2454-1354

Volume – 8; Issue - 2; Year – 2022; Page: 2367 – 2376

DOI: 10.22192/lsa.2021.8.2.4

Research Article

SYNERGISTIC INTERACTION OF ASCORBIC ACID AND EDTA AGAINST *Escherichia coli* AND *Pseudomonas aeruginosa* ISOLATED FROM RAW MILK

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Abstract

The goal of this study was to see how ascorbic acid and EDTA worked together to fight *Escherichia coli* and *Pseudomonas aeruginosa* that were found in raw milk in Al Qasim city, Egypt. Al Jawthar and Fayyadieh are two areas in Al Qasim Province that have a lot of raw milk. We took 40 samples from Al Jawther, and 40 samples from the other area (10 samples/week). All of the samples were taken to the lab in an icebox for the analyzer. As a final confirmation, Eosin methylene blue (EMB), nutrients, violate red bile agar (VRB), and biochemical tests were used to identify *Escherichia coli* and *Pseudomonas aeruginosa*, respectively. This present study found that out of the 90 samples tested, 29 (32.2 %) were positive for *Escherichia coli* growth, 18 (20 %) were from the village of Fayadieh and 11 (12.5 %) were from the village of Al Jawther, while 17 (18.8 %) were positive for *Pseudomonas aeruginosa* growth, with 10 (11.1 %) being from the village of Fayadieh and 7 (7.7 %) being from Al Jawther. Ascorbic acid and EDTA were tested in various concentrations against *Escherichia coli* and *Pseudomonas aeruginosa* in this study. A 20 - 26 mm inhibition zone is visible at 1 % and 2 % concentrations of *Pseudomonas aeruginosa*, while an inhibition zone of 16 - 24 mm is visible at EDTA concentrations. For *Pseudomonas aeruginosa*, the inhibition zone appeared at 1 % and 2 % concentrations from (22 mm to 23 mm), while for *Escherichia coli*, the inhibition zone appeared at the same concentration from (13 mm to 16 mm). At the same concentration, EDTA-ascorbic acid inhibited *Pseudomonas aeruginosa* (12 mm to 25 mm) and *Escherichia coli* (19 mm to 21 mm) at the same time, according to the study results. Ascorbic acid and EDTA are antibacterial and play an important role in food preservation and delaying the growth of microorganisms, according to the findings of this research.

Article History

Received : 30.01.2022
Revised : 28.02.2022
Accepted : 18.03.2022

Key words: Raw milk, EDTA, Ascorbic acid and Bacterial spoilage

1. Introduction

Microorganisms can get into milk that comes from healthy animals during or after they are milked, but they can't get into the milk itself (Makerere University, 2011; Velázquez-Ordoez *et al.*, 2019). From the air, you can see Milkier, the west, handlers, and the medicines that are used to treat the animals (Swai and Schoonman, 2011). Depending on the strain, some of these bacteria can be harmful in milk, while others can be harmful to humans when milk has gone bad (Bukuku, 2013). One example is *Escherichia coli*

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O157:H7, which is commonly found in milk. Emerging new milk-pathogen bacterial pathogens such as *Escherichia coli* have recently been reported to have a variety of serious health consequences (Paswan and Park, 2020).

In part due to their ability to survive in the presence of chilling storage, *Pseudomonas* spp. are among the most common bacteria involved in the spoilage of milk and dairy products during this period (Martin *et al*., 2011). In addition, some of these strains released heat-stable extracellular lipases, proteases, and lecithinases, which can cause milk and dairy products to spoil. These enzymes can also kill the bacteria that make them. Bacteria and other enzymes can make milk ingredients go bad, which can make dairy products last less long on the shelf (Hammad, 2015).

The chelating agent Ethylene diamine tetraacetic acid (EDTA) has been used a lot in a lot of different food products to keep things like degrading processes and other oxidation caused by metal ions from happening (Branen and Davison, 2004; Kopermsub *et al*., 2011). When Mg+2 and Ca+2 are removed from outer membrane locations by divalent cation chelator EDTA, lipopolysaccharides, proteins, and other cell contents are released. (Chaudhary *et al.*, 2013). As a more specific example, sorbets are a natural antioxidant and antibacterial agent that is synthesized by plants and most animals (excluding humans, primates, and guinea pigs) and can be incorporated into a wide variety of foods **(**Isela *et al.*, 2013). Sorbates (ascorbic acid) and their water-soluble salts are effective against yeasts and molds, as well as a wide range of bacteria. Sorbate has been shown to inhibit a wide range of bacterial *Escherichia coli* (Koodie and Dhople, 2001). Microorganisms can be significantly influenced by factors inherent to the food, such as the concentration of NaCl and the pH value, in addition to antimicrobials approved for use in foods (Doores, 1993). The objectives of the study were to investigate the synergistic effects of ascorbic acid alone and in combination with EDTA against *Escherichia coli* and *Pseudomonas aeruginosa*, which were isolated from raw milk in Al Qasim city.

2. Materials and Methods

Sample collection

Forty raw milk samples were collected randomly from different locations of Al Qasim (Al Jawthar, Al Fayyadieh) using a sterile aseptic glass jar and cool transported using ice and icebox to laboratory examination in Al-Qasim University, Veterinary Medicine **(**Khudhier, 2011).

Isolation and identification of bacteria

Detection of *Pseudomonas aruginosa*

Take 1 ml of MacConkey-cultured samples, and mix them with water. Plates were incubated at 37 °C for the duration of the experiment. Colonies that were not fermentative appeared pale on agar after incubation. A pure culture of these colonies was obtained by sub-culture on Brain heart infusion agar for further identification purposes (Garrity *et al*., 2005).

Detection of *Escherichia coli*

Escherichia coli was counted on MacConkey agar that had been incubated at 37 degrees Celsius for 24 - 48 hours. Suspected colonies were confirmed by EMB and Indole, Methyl red, Voges-Proskauer, Citrate (IMViC) tests (Jamshidi *et al*., 2008).

Preparation of Stock solutions

Preparation of Ascorbic acid

The stock solution of ascorbic acid was prepared by dissolving 0.25 %, 0.50 %, 0.75 %, 0.1%, and 2 % of the acid in enough sterile distilled water to make the final volume of the solution of (10 ml) (Doughari *et al*., 2007).

Preparation of EDTA

Prepared by adding 0.25 %, 0.50 %, 0.75 %, 1 % and 2 % of Disodium ethylene diamine tetraacetic acid to 10 ml of D.W. Stirring vigorously on a magnetic stirrer (Borowicz *et al.*, 2016).

Determination of Minimum Inhibitory Concentration (MIC)

The tube dilution method was used to determine the inhibitory activity. Preparation and sterilization of stock solutions of ascorbic acid and EDTA by filtration are both standard operating procedures. Afterwards, each tube was inoculated with 0.1 ml of an overnight bacteria culture, and the tubes were incubated at 37 ºC for 24 hrs at this temperature. Turbidity in the tubes and growth in the Petri dishes suggest a successful outcome (Jesline *et al.*, 2014).

Determining Effects of Ascorbic acid in Combination with EDTA

After preparing the Ascorbic acid solution, the desired concentration of EDTA (0.25 %, 0.50 %, 0.75 % and 2 %) was added. The resolidified Mueller Hinton agar (MHA) plates were well-bored using a sterile cork borer with an 8.0 mm diameter. Ascorbic acid and EDTA inhibition zones against bacteria were determined using the Well diffusion assay and a 25 µl final solution in the agar well diffusion (per the recommendations of the National Committee for Clinical Laboratory Standards) (NCCLs, 2001).

4. Results and Discussion

Isolation and Identification of *Escherichia coli* and *Pseudomonas aeruginosa*

The raw milk samples were taken from two different villages near Al Qasim, one of which was Al Jawthar and the other was Fayyadieh. Each village had 10 samples taken at random every week for a total of 90 samples. *Escherichia coli* and *Pseudomonas aeruginosa* were isolated and identified from the raw milk samples. The results of this study show that out of the 90 samples of *Escherichia coli* growth that were found, 18 (20 percent) came from Fayyadieh village and 11 (12.5 percent) came from Al Jawther village. This study is in line with (Daher, 2013), which found that more raw milk samples were contaminated with *Escherichia coli* when they were taken from seven villages around Baghdad province. In this study, 18 of 35 raw milk samples were contaminated with *Escherichia coli* (Khudhier, 2011) while collecting raw milk samples from dairy farmers around Baghdad, researchers found *Escherichia coli* isolates 15 (20 %) in the samples, according to a report (Shunda *et al.*, 2013). Only 44.4 % of the samples from Mekelle, Ethiopia were positive for *Escherichia coli*. However, 57 % of the 100 raw milk samples tested were positive for this bacteria, according to Soomro *et al*. (2002). Reta *et al*. (2016) revealed that the level of contamination with *Escherichia coli* is 70 (58 %) in raw milk sample in Ethiopia.

Overall 90 sample was collected 17 (18.8 %) isolate positive of *Pseudomonas aeruginosa*, 10 (11.1 %) isolate from Fayyadieh village and 7 (7.7 %) isolate from Al Jawther village (Scaccabarozz *et al.*, 2015) who reported 12 (50 %) isolates of *Pseudomonas aeruginosa* from raw milk. Keskin and Ekmekci (2007) reported that 21 % of the isolates from milk were *Pseudomonas aeruginosa* (Swetha *et al*., 2017) and found that the 19 (15.2) positive samples for *Pseudomonas aeruginosa* isolate from raw milk. Microbial contamination in the milk market value chain can be caused by a diseased cow, improper milking practices, poor personal hygiene, dirty utensils and/or milking equipment, poor storage conditions, and a lack of pure water supply, according to evidence (Berhe *et al.*, 2020). As a result, enhancing animal health, environmental hygiene, dairy farming methods, milk handling, transportation, and storage practices are essential to prevent pathogenic microorganism contamination of milk (Oliver *et al.*, 2005).

The interaction of EDTA and Ascorbic acid against *Pseudomonas aeruginosa*

Our study showed the effect of Ascorbic acid alone and combined with EDTA against bacteria isolate in different concentrations (0.25 %, 0.50 %, 0.75 %, 1 % and 2 %). The results show that the inhibition zone against *Pseudomonas aeruginosa* appears (11 mm, 14 mm, 20 mm, 22 mm and 23 mm) at a concentration of 0.25 %, 0.50 %, 0.75 %, 1 % and 3 % respectively when used ascorbic acid alone. While none any inhibition zone around well as concentration (0.25 %, 0.50 % and 0.75 %)

against *Escherichia coli* only the inhibition zone (13 mm to 19 mm) at the concentration (1 % and 2 %) as in Table - 2 and 3.

The results show that using EDTA against bacterial isolation alone at a different concentration did not influence bacterial growth in the concentration (0.25 %, 0.50 %, 0.75 %) only the inhibition zone (20 mm to 26 mm) against *Pseudomonas aeruginosa* and 16 mm to 24 mm against *Escherichia coli* at the concentration (1 % and 2 %) respectively. Our study showed that the effects of EDTA – Ascorbic acid combination against isolates indicated that inhibition zone (11 mm, 15 mm, 18 mm, 21 mm and 25 mm) against *Pseudomonas aeruginosa* at a concentration of 0.25 %, 0.50 %, 0.75 %, 1 % and 2 %. While non any inhibition zone present in concentrations (0.25 %, 0.50 % and 0.75 %) only at concentration (1 % and 2 %) the inhibition zone (19 mm to 23 mm) (Borowicz *et al.,* 2016) who reported that the antimicrobial activity of EDTA was strongly dependent on the concentration of the active compound and increased with increasing content of compound; also found did not influence on bacterial growth in the amount of 0.25 % and 0.50 %. While the bacterial isolation is sensitive to EDTA in concentration 1% this study agrees with our study against *Escherichia coli* and *Pseudomonas aeruginosa*. EDTA has a more complex inhibition-concentration profile against *Pseudomonas aeruginosa* found that low concentrations of EDTA caused the formation of convoluted surfaces, but no lyses (Lambert *et al.,* 2004; Porras-Gómez *et al.,* 2018) reported that EDTA concentrations with a maximum of 125 µM were growth inhibition by EDTA alone. The minimum concentration of EDTA is 1.957 µM that does not inhibit bacterial growth. EDTA has antimicrobial properties against various Gram-negative bacteria (Reidmiller, *et al.,* 2006).

Food additives and preservatives like ascorbic acid are commonplace (Lee, *et al.,* 2003), as well as an important antioxidant, applied in pharmaceutical and cosmetic industries (Tabak, *et al.,* 2003; Verghese, *et al.,* 2017) A new class of topical antimicrobial agents could benefit from Ascorbic acid's ability to inhibit bacterial growth. Vitamin C's inhibitory activity may have novel clinical applications, including topical antimicrobial applications. Beth *et al.* (2004) discovered that the effectiveness of organic acids as antimicrobials varies significantly depending on their concentration, pH, molarity, and the concentration of the non-dissociated form of the acid. Jageethadevi *et al.* (2011) reported that each the increase concentration of chemical preservation increase the inhibition zone. Nada (2005) found that inhibition zone that less 4 mm is resistance, 4 mm to 12 mm are intermediates while inhibition zone more 12 mm sensitive to chemical preservations, inhibition zone depends on Gram reaction of preservative. The Gram positive is more sensitive than Gram negative to chemical preservatives (Oladopo *et al.,* 2014). Gram negative bacteria (cell wall) composed of peptidoglycan, its synthesis or structure lead to the loss of this composed, followed by bacterial death (Willey *el al.,* 2011). Neutralized ascorbic acid is found to exert a strong bactericidal action on *Pseudomonas aeruginosa*; Ascorbic acid alters the cell surface to render it increasingly permeable other antibacterials (Rawal, 1978).

EDTA helps Gram-negative bacteria release LPS from their Outer membrane (OM) by chelating calcium and magnesium salts that interact with their LPS layer (Sangcharoen *et al.,* 2017). EDTA, as well as a number of organic acids and their salts, are chelating agents, as previously reported (Suksathit and Tangwatcharin, 2013). Davidson and Branen (2005) discussed the antimicrobial efficacy of organic acids and noted that the efficacy of an organic acid on microorganism inhibition is dependent on its pKa value, which is the pH at which 50 % of the acid is dissociated. An organic acid can only pass through the cell membrane of a microorganism if it is in its un dissociated form, and at lower pH levels, there is a greater concentration of the un dissociated form of the acid present. A high pKa value for an organic acid indicates that it contains a greater amount of acid in its undissociated form, and thus has a greater antimicrobial efficacy.

Table - 1: Prevalence contamination of Raw milk in a different villages in Al Qasim city

Place	*Escherichia coli*		*Pseudomonas aeruginosa*	
	No. of positive	Isolate %	No. of positive	Isolate %
Al Jawther	11	12.5 %	7	7.7%
Fayyadieh	18	20 %	10	11.1%
Total (90)	29	32.2 %	17	18.8%

Table - 2: Antibacterial activity of Ascorbic acid, EDTA and combined against *Escherichia coli*

Concentration	Size of inhibition zone mean(±) diameter milliliter		
	Escherichia coli		
	EDTA	Ascorbic acid	EDTA- Ascorbic acid
0.25 %	0	0	0
0.50 %	0	0	0
0.75 %	0	0	0
1 %	16	13	19
2 %	24	19	23

Table - 3: Antibacterial activity of ascorbic acid, EDTAand combined against *Pseudomonas aeruginosa*

Concentration	Size of inhibition zone mean(±) diameter milliliter		
	Pseudomonas aeruginosa		
	EDTA	Ascorbic acid	EDTA- Ascorbic acid
0.25 %	0	11	11
0.50 %	0	14	15
0.75 %	0	20	18
1 %	20	22	21
2 %	26	23	25

4. Conclusion

Ascorbic acid and EDTA were antibacterial andplayed role important in food preservation and delaying the microbial growth.

Acknowledgements

Would like to express their gratitude to assist. Prof. Dr. Abed Al Karem Altae, Head, Department of Microbiology/ College Veterinary Medicine/ Al Qasim Green University. Also, would like to thank theHead of the Department Veterinary Public Health /College Veterinary Medicine/University of Al Qasim Green.

5. Reference

1) Adams, D. M; Baruch, J. T. and Speck, M. L.(1975). Heat resistant proteases produced in milk by psychotropic bacteria of dairy origin. Journal of Dairy Science 58:828.
2) Axelson, L. (1998). Review of the sensitivity of different Food borne pathogens to fermentation food control, U.K**).** Lactic acid bacteria: classification and physiology of lactic acid bacteria, Microbiology and Functional Aspects.

Salminen, S. and Weight, A. (Eds), Marcel Decker Inc., New York.

3) Bearson, S.; Bearson, B. and Foster, J. (1997). Acid stress sesponses in enterobacteria. FEMS Microbial. Lett. 147: 173-180.
4) Bell, C. and Kyriakides, A. (1998). Escherichia coli: A practical approach to the organism and its control in foods. 1st ed. London, United King: Blackie Academic.
5) Berhe, G.; Wasihun, A. G.; Kassaye, E., and Gebreselasie, K. (2020). Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, northern Ethiopia. *BMC Public Health*, *20*(1), 1-8.
6) Beth, C.; Mark, C. and Zuoxing, *J.* (2004) .An overview of antimicrobial ingredient .Journal of Food Safety. 10, 24 - 25.
7) Borowicz, J., Kasperski, K., Gwiazdowska, D., Tichoniuk, M., Wojciechowska, P., and Juś, K. (2016). Antibacterial activity of chemical compounds used for active packaging.
8) Boughattas, S. and Salehi, R. (2014). Molecular approaches for detection and identification of foodborne pathogens. Journal of Food Quality and Hazards Control. 1: 1-6.
9) Branen J.K. and Davidson P.M. (2004). Enhancement of nisin, lysozime, and monolaurin antimicrobial activities by ethylene diamine tetraacetic acid lactoferrin. International Journal of Food Microbiology. 90: 63-74.
10) Bukuku, J.N. (2013). Awareness of health risks as a result of consumption of raw milk in Arusha City and Meru District, Tanzania. Unpublished dissertation for award of MSc. degree at Sokoine University of Agriculture, Morogoro, Tanzania. pp 1 - 89.
11) Caro, I. and García-Armesto, M. (2007) . Occurrence of Shiga toxin-producing *Escherichia coli* in a Spanish raw ewe's milk cheese. Int. J. Food Microbiol., 116:410-413.
12) Chao G.; Zhou X.; Jiao X.; Qian X. and Xu, L. (2007). Prevalence and antimicrobial resistance of foodborne pathogens isolated from food products in China. Foodborne Pathogen and Disease. 4: 277 - 284.
13) Cursino, L.; Chartone-Souza, E. and Nascimento, A. M.(2005). Synergic Interaction between Ascorbic Acid and Antibiotics against *Pseudomonas aeruginosa*. Brazilian Archives of Biology and Technology . 48(3) : pp. 379-384
14) Daher, N. A. (2013).The synergistic bacteriocidal effect of bacteriocin and pressurization against *E. coliO157:H7* in raw milk . M.SC. Thesis, Collage of Veterinary Medicine ,University of Baghdad.
15) Davidson, P.M.; Post, L.S.; Branen, A.L. and Mccurdya, R. (1983). Naturally occurring and miscellaneous food antimicrobials. In Antimicrobials in Foods ed. Brannen, A.L. & Davidson, P.M. pp. 371419. New York: Marcel Dekker.
16) Davidson, P.M. ; Sofos,J.N and Branen, A. L. (2005). Antimicrobials in Food, CRC Press, Boca Raton, FL, USA, pp. 1-10.
17) DeWit, J. N. (1989).Functional properties of whey proteins in Development in Dairy Chemistry. 4. Functional milk proteins. P.F. Fox (Ed.) Elsevier Applied Science. London. pp. 339-367.
18) Doores, S. (1993). Organic acids. In: Davidson, M.P., Branen, A.L. (Editors). Antimicrobials in Foods. 2nd edition. Marcel Dekker, Inc., New York. pp: 95–135.
19) Doughari, J.H.; Elmahmood, A. M. and Manzara, S.(2007). Studies on the antibacterial activity of root extracts of Carica papaya L. Afr. J. Microbial Res. 7 : 37- 41.
20) Dowd, M. T and Dent, A.(1937). Elements of Food and Nutrition Chapman and Hall Limited. New York. pp 130-133.

21) FDA (1998). Milk In: "Code of Federal Regulations. Title 21. Section 131.110. U.S. Govt. Printing Office. Washington.
22) Fratamico, P. M. and Smith, J.L. (2006): *Escherichia coli* infections. In: Riemann, H.P., Cliver, D.O. (3rd): Food-borne infections and intoxications. Florida: Elsevier Inc. Academic Press. pp. 205-208.
23) FSA (Food Science Australia) (2002): How useful are microbiological criteria for fresh meat? In: Meat technology update, February, 2002. Paper in Newsletter.
24) Garrity, G. M.; Bell, J.A. and Lilburn, T. (2005): Order IX. Pseudomonadales. Pages 323–371 in Bergey's Manual of Systematic Bacteriology. Vol. 2. D. J. Brenner, N. R. Krieg, and G. M. Garrity, ed. Springer, New York, NY.
25) Godefay, B. and Molla, B.(2000).Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa. Berl. Munich. Tierarzil wschr. 113, PP. 1-3.
26) Hammad, A. M. (2015). Spoilage potential of *pseudomonas spp*. isolated from domiati cheese. Assiut. Vet. Med. J. 147(61).
27) Hennekinne, J.A.; De Buyser, M. L. and Dragacci S. (2012). *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiology Reviews*. 36: 815 - 836.
28) Holzapfel, W.; Geisen, R. and Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol**.,** 24: 343-362.
29) International Organization for Standardization (ISO) (2005). Standard protocol (3rd): Microbiology of food and animal feeding stuff – Horizontal method for the detection and enumeration of presumptive Escherichia coli - Most probable number technique. Switzerland.
30) Isabelle Hininger; Robert Waters; Mireille Osman; Catherine Garrel; Karen Fernholz; Anne Marie Roussel and Richard A. Anderson(2005). Acute prooxidant effects of vitamin C in EDTA chelation therapy and long-term antioxidant benefits of therapy. Volume 38, Issue 12, 15 June , Pages 1565-1570.
31) Isela, S. N. R.; Sergio, N.-C.; José, M.-S. J.; Rene, H-D. and Claudio, C-R . (2013). Ascorbic Acid On Oral Microbial Growth and Biofilm formation. The Pharma. Innovation – Journal. Vol. 2 : 4.
32) Jageethadei, A. Saranraj, P and Ramya, N. (2012). Inhibitory effect of chemical preservatives and organic acid on the growth of bacterial pathogens in poultry chicken, Asian ,J. Biochem. Pharm. Res. 1(2): 1-9.
33) Jamshidi, A.; Bassami, M. R. and Rasooli, M. (2008). Isolation of *Escherichia coli O157:H7* from ground beef samples collected from beef markets, using conventional culture and polymerase chain reaction in Mashhad, northeastern Iran. Iranian Journal of Veterinary Research, Shiraz University, Vol. 9, No. 1, Ser. No. 22.
34) Jawetz, E; Melinick, J. L. and Adelberg, E. A(1980). Review of Medical Microbiology, 14PthP (eds.), Middle East Edition 1980.
35) Jay, J. M.; Loessner, M.J. and Golden D.A. (2005). Modern food microbiology. 7th edition. Springer Science+ Business Media, Inc, New York. pp: 304-325.
36) Jeffrey S. Reidmiller ; Wayne L. Smith; Mary M. Sawyer; Bennie I. Osburn; Jeffery L. Stott And James S. Cullor(2006). Antimicrobial Properties of the Chelating Agent EDTA on Streptococcal Bovine Mastitis Isolates. Journal of Food Protection, Vol. 69, No. 6, Pages 1460–1462.
37) Jesline, A.; John, N. P.; Vani, C. and Nurugan, S. (2014). Antimicrobial activity of zinc and titanium dioxide nanoparticles against biofilm –producing methicillin resistant *Staphylococcus aureus*. Appl. Nanosci.13:1-6.

38) Karim, H. A. (2008). Effect of Tris-EDTA and ascorbate in increasing antibiotic activity against bacteria isolated from Otitis Media. A Thesis. College of Science of Al-Nahrain University.
39) Keskin, D. and Ekmekci, S. (2007). Investigation of the incidence of *Pseudomonas* sp. in foods. Hacettepe Journal of Biology and Chemistry. 35(3): 181-186.
40) Khudhier, S.Z.(2011). Antibacterial activity of *L. acidophilus* bacteriocin against *E. coli O157:H7* in raw milk . PhD thesis, College of Veterinary Medicine ,University of Baghdad.
41) Kivaria, F.M.; Noor dhuizen, J.P.T.M. and Kapaga, A.M. (2006a). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar ES Salaam region, Tanzania.Tropical Animal Health Production 38: 185 - 94.
42) Koodie, L.and Dhople, A.M. (2001). Acid tolerance of *Escherichia coli O157:H7* and its survival in apple juice. *Microbios*. 104: 167– 175.
43) Kopermsub, P.; Mayen, V. and Warin C. (2011). Potential use of nisosomes for encapsulation of nisin and EDTA and their antibacterial activity. *Food Research International*. 44: 605-612.
44) Lambert, R.J.W.; Hanlon, G.W. and Denyer, S.P. (2004)**.** The synergistic effect of EDTA/antimicrobial combinations on Pseudomonas aeruginosa. Journal of Applied Microbiology , 96, 244 – 253.
45) Lambert, R.J.W.; Hanlon, G.W. and Denyer, S.P. (2004). The synergistic effect of EDTA/antimicrobial combinations on *Pseudomonas aeruginosa.* Journal of Applied Microbiology *;* 96, 244 – 253.
46) Lee, J. B.; Ahn, J. and Kwak, H. S. (2003), Microencapsulated ascorbic acid for milk fortification. Arch. Pharm. Res., 26, 575-80.
47) Makerere University, School of Veterinary Medicine,(2011).Dairy Products Quality and Safety Module. 38pp.
48) Marilyn Porras-Gómez; José Vega-Baudrit; Fernando García; Santiago Núñez-Corrales and Sergio Madrigal-Carballo (2018). Evaluation of the Synergistic Effect of EDTA-Functionalized Chitosan Nanoparticles on Imipenem Delivery in *Pseudomonas aeruginosa* Carbapenem-Resistant Strain AG1. Journal of Biomaterials and Nano biotechnology. 9: 64-78.
49) Mosalagae, D.; Pfukenyi, D.M. and Matope, G. (2011). Milk producer's awareness of milk-borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. Tropical Animal Health and Production 43: 733 – 739.
50) Nanda, M. (2005). Determination of zone of inhibition in: clinical microbiology 1st Ed. Jaype Brothers medical publishers, New Delhi.
51) Oladapo, A. S. ; Akinyosoye, F. A. and Abiodum,O. A.(2014). The inhibition effects of different chemical food preservative on the growth of selected food borne pathogenic bacteria. African journal of microbiology research. Vol 8(14).pp 1510-1515.
52) Oliver, S. P.; Jayarao, B.M. and Almeida, R.A. (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis; 2:115–29.
53) Östling, C and Lindgren, S. (1993). Inhibition of enterobacteria and *Listeria* growth by lactic, acetic and formic acids. J. Appl. Bacteriol., 75: 18-24.
54) Parekh, T.S. and Subhash, R. (2008). Molecular and bacteriological examination of milk from different milch animals with special reference to Coliforms. Current Research in Bacteriology 1(2): 56 - 63.
55) Paswan, R., and Park, Y. W. (2020). Survivability of Salmonella and Escherichia coli O157: H7 pathogens and food safety concerns on commercial powder milk products. *Dairy*, *1*(3), 189-201.

56) Radiostitis, O. M.; Blood, D.C. and Gay, C.C(1994).Veterinary Medicine Text Book of the Disease of cattle, Sheep, Pigs, Goats and Horses, 8 the Edition, Bailliere Tindall,PP.64-575.
57) Rasmussen, C.N.; Wang, X.; Leung, S.; Andrae-Nightingale, L.M.; Schmidt, S.J. and Engeseth, N.J. (2008). Selection and use of honey as an antioxidant in a French salad dressing system. *Journal of Agricultural and Food Chemistry* 56, 8650–8657.
58) Raut, J. S. and Karuppayil, S. M. (2014). A status review on the medicinal properties of essential oils. Industrial Crops and Products, vol. 62, pp. 250–264.
59) Rawal, B.D.(1978). Bactericidal Action of Ascorbic Acid on *Pseudomonas aeruginosa*: Alteration of Cell Surface as a Possible Mechanism. Chemotherapy; 24: 166 – 171.
60) Reta, M. A.; Bereda, T. W. and Alemu A. N. (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia.International Journal of Food Contamination.
61) Sangcharoen, N., Klaypradit, W., and Wilaipun, P. (2017). Antimicrobial activity optimization of nisin, ascorbic acid and ethylenediamine tetraacetic acid disodium salt (EDTA) against Salmonella enteritidis ATCC 13076 using response surface methodology. *Agriculture and Natural Resources*, *51*(5), 355 - 364.
62) Scaccabarozzi, L.; Leoni, L.; Ballarini, A.; Barberio, A.; Locatelli, C.; Casula A.; Bronzo, V.; Pisoni ,G.; Jousson, O.; Morandi, S.; Rapetti, L.; García-Fernández, A. and Moroni, P.(2015). *Pseudomonas aeruginosa* in Dairy Goats: Genotypic and Phenotypic Comparison of Intramammary and Environmental Isolates. PLoS One. 25;10(11).
63) Shunda, D.; Habtamu, T . and Endale, B.(2013). Assessment of Bacteriological Quality of Raw Cow Milk at Different Critical Points in Mekelle, Ethiopia. International Journal of Livestock Research.
64) Soomro, A.H.; M.A. Arain; M. Khaskheli and B. Bhutto (2002). Isolation of *Escherichia coli* from raw milk and milk products is relation to public health sold under market condition at Tandojam Pak. J. Nutr., 1: 151- 152.
65) Suksathit, S. and Tangwatcharin, P. (2013). Activity of organic acid salts in combination with lauric arginate against *Listeria monocytogenes* and *Salmonella* Rissen. Sci. Asia, 39 : pp. 346-355.
66) Swai, E.S. and Schoonman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. Asian Pacific Journal of Tropical Biomedicine 1(3): 217 - 222.
67) Swetha, C. S. ; A. Jagadeesh, A. B. ; Venkateswara, K .; Bharathy, S.; Supriya, R. A. and Madhava, R. T. (2017). A study on the antimicrobial resistant patterns of *Pseudomonas Aeruginosa* isolated from raw milk samples in and around Tirupati, Andhra Pradesh. Asian J. Dairy and Food Res, 36(2) : 100-105.
68) Tabak, M.; Armon, R.; Rosenblat, G.; Stermer, E. and Neeman, I. (2003). Diverse effects of ascorbic acid and palmitoyl ascorbate on Helicobacter pylori survival and growth. FEMS Microbiol Lett., 224, 247-253.
69) Todar, K. (2005): Ken Todar's Microbial World. University of Wisconsin–Madison. In (ed.): Bacteriology at UW-Madison. Lectures and readings from introductory microbiology courses at the University of Wilson's in Madison.
70) Velázquez-Ordoñez, V., Valladares-Carranza, B., Tenorio-Borroto, E., Talavera-Rojas, M., Varela-Guerrero, J. A., Acosta-Dibarrat, J.and Pareja, L. (2019). Microbial contamination in milk quality and health risk of the consumers of raw milk and dairy products. In *Nutrition in Health and disease-our challenges Now*

and Forthcoming time. London, UK: IntechOpen.

71) Verghese, J. R. ; Mathew, K.S. and David, A. (2017). Antimicrobial activity of Vitamin C demonstrated on uropathogenic *Escherichia coli* and *Klebsiella pneumonia*. *Journal of Current Research in Scientific Medicine*, 3(2): 88 – 93.

72) Willey, J. O.; Sherwood ,L. M. and Woolverton, C. J. (2011). Prescott microbiology: in food intoxication and infection. McGraw Hill Publish. 8th Ed pp. 1015-1016.

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DOI Number	**DOI: 10.22192/lsa.2021.8.2.4**
Thomson Reuters Researcher ID	**L – 5547 – 2016**
ISI Impact Factor	**4.206**

How to Cite this Article:

Mussa M. Alkhatib, Mohammed Khadim Wali and Entesar Hussain Madi. (2022). Synergistic Interaction of Ascorbic acid and EDTA against *Escherichia coli* and *Pseudomonas aeruginosa* Isolated from Raw Milk. *Life Science Archives*, 8(2): 2367 – 2376.

DOI: 10.22192/lsa.2021.8.2.4