Espelho da Prova de proficiência em Língua Inglesa – Agrárias e Biológicas

EVALUATION OF ANTIBACTERIAL ACTIVITY OF VITAMIN C AGAINST HUMAN BACTERIAL PATHOGENS[1]

S. Mumtaza, S. Mumtaza, S. Alia, H. M. Tahira , S. A. R. Kazmib, T. A. Mughalc and M. Younas[2]

Abstract

Now a day's multidrug resistance phenomenon has become the main cause for concern and there has been an inadequate achievement in the development of novel antibiotics to treat the bacterial infections. Therefore, there is an unmet need to search for novel adjuvant. Vitamin C is one such promising adjuvant. The present study was aimed to elucidate the antibacterial effect of vitamin C at various temperatures (4°C, 37°C and 50°C) and pH (3, 8, and 11), against Gram-positive and Gram-negative bacteria at various concentrations (5-20 mg/ml) through agar well diffusion method. Growth inhibition of all bacterial strains by vitamin C was concentration-dependent. Vitamin C significantly inhibited the growth of Gram-positive bacteria: Bacillus licheniformis (25.3 ± 0.9 mm), Staphylococcus aureus (22.0 ± 0.6 mm), Bacillus subtilis (19.3 ± 0.3 mm) and Gram-negative bacteria: Proteus mirabilis (27.67 ± 0.882 mm), Klebsiella pneumoniae (21.33±0.9 mm), Pseudomonas aeruginosa (18.0 ± 1.5 mm) and Escherichia coli (18.3 ± 0.3 mm). The stability of vitamin C was observed at various pH values and various temperatures. Vitamin C showed significant antibacterial activity at acidic pH against all bacterial strains. Vitamin C remained the stable at different temperatures. It was concluded that vitamin C is an effective and safe antibacterial agent that can be used in the future as an adjunct treatment option to combat infections in humans.

Keywords: agar well diffusion method, antibacterial activity, vitamin C, Pseudomonas aeruginosa

Introduction

Vitamin C is an important antioxidant, free radical scavenger, pro-oxidant, and an antibacterial molecule that can modify the antimicrobial activity of various antibiotics as well as significantly declines the adversative effects of reactive species (Kwiecinska-Pirog et al., 2019). Among the most common infections, urinary tract infections (UTIs) especially among women are seen around the world. Various bacterial strains such as Klebsiella pneumoniae and E. coli have antibiotic resistance which results in more complications occur (Ahmed et al., 2019). Therefore, it is needed to treat various infections including urinary tract infections and vitamin C is one of the cheap alternatives that have no adverse effects and easily available (Verghese et al., 2017). Hong et al. (2016) stated that high concentrations of vitamin C, particularly, possess immunomodulatory functions, and antimicrobial properties, therefore, decreasing the risk

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[2] Government College University, Department of Zoology, Applied Entomology and Medical Toxicology Laboratory, Lahore, Pakistan bGovernment College University, Department of Chemistry, Lahore, Pakistan c Department of Zoology, Women University of Azad Jammu and Kashmir, Bagh, Pakistan dUniversity of Lahore, Institute of Molecular Biology and Biotechnology, Lahore, Pakistan

of infections. The antibacterial effect of vitamin C particularly, L- ascorbic acid has been found against various pathogenic organisms including Bacillus subtilis, Corynebacterium diphtheria, Enterococcus faecalis, and Staphylococcus aureus (Isela et al., 2013). It also prohibits the mycobacterium tuberculosis and Helicobacter pylori, which is responsible for carcinoma (Vilchèze et al., 2013). The pharmacological use of ascorbic acid is supposed to improve the role of the immune system and a precarious basal meditation of vitamin C is crucial for an average and well-functional host resistance mechanism (Van Gorkom et al., 2018). Humoral and cellular immune responses might be reduced due to the deficiency of vitamin C (Jeong et al., 2014). Moreover, in humans and other experimental in vivo models, the influence of ascorbic acid on various immune cell populations has been revealed (Van Gorkom et al., 2019). In experimental studies, vitamin C treatment enhanced and promoted lymphocyte proliferation, natural killer cell activities, and chemotaxis besides its anti-inflammatory properties (Hemila, 2017). It is also observed that concentrations of vitamin C are 10- to 100-fold higher in immune cells e.g., leukocytes than those measured in the plasma (Strohle et al., 2011). Furthermore, hydroxylase enzymes require vitamin C as a cofactor to transcription of gene, cell signaling of immune system cells and the action of the hypoxia-inducible factors (Kuiper and Vissers, 2014). Vitamin C possesses an inhibitory influence against pathogens (Helicobacter pylori), in the gut, which is a recently recognized causal agent of sores (Namiot et al., 2020). Woo et al. (2010) reported that ascorbic acid could kill the strains of mycobacterium tuberculosis that are resistant to most other antibiotic drugs. In various studies, fruit juices containing vitamin C were used as antimicrobials that reveal that vitamin C act as an antibacterial agent (Opara et al., 2009).

The purpose of conducted research was to elucidate the antibacterial potential of vitamin C (L-ascorbic acid) against Gram-positive and Gram-negative bacterial pathogens and optimization of stability at different pH values and temperature.

Materials and Methods

Bacterial strains and culture media

Gram-positive {B. licheniformis (FCBP-SB-0019), B. subtilis (FCBP-SB-0223), S. aureus (FCBP-WB-0260), and Gram-negative bacterial strains K. pneumoniae (FCBP-PB-0047), pseudomonas aerguginosa (FCBP-PB-0083), E. coli (FCBP-SB-0011), and P. mirabilis (FCBP-PB-0043)} were taken from Institute of Agricultural Sciences, University of The Punjab, Lahore, and the Microbiology Department, Government College University, Lahore, Pakistan. Nutrient broth (CM0001, OXOID LTD., and Agar CAS Nr: 9012-36-6 (Sigma- Aldrich Company) was used as a culture medium for the growth of bacteria. Vitamin C (L-ascorbic acid, CAS Nr. 50-81-7) with molar mass (176.12 g/mol) was purchased from Sigma Aldrich Company. HCL (CH_3COOH, M =60.05 g/mol, Merck KGaA Darmstadt, Germany) was used. Sodium hydroxide (NaOH, CAT No. S5881, Sigma-Aldrich, Saint Louis, MO 63103, USA) was used. Erythromycin with (Mfg. Lic. No. 000124) was purchased from Indus Pharma (Pvt. Ltd), and used as a positive control.

Antibacterial test

The antibacterial activities of vitamin C were evaluated via agar well diffusion method against Gram-positive (B. licheniformis, S. aureus and B. subtilis and Gram-negative bacteria P. mirabilis, P. aeruginosa, K. pneumoniae, and E. coli) (Hwang et al., 2020). Briefly, freshly prepared bacterial growth or culture media was poured into the sanitized petri dishes in a laminar airflow. After solidifying, the petri dishes were incubated for 24 h at 37°C. Then 50 µl of nutrient broth containing test organisms was added into plates through a micropipette and spread over the whole petri plates with a spreader. Petri plates were air-dried under sterile conditions for 10 min and wells (5 mm diameter) were formed. In the remaining wells 50 µl of test solution (vitamin C) with various concentrations (5 mg, 10 mg, and 20 mg /ml) was added via micropipette. Erythromycin (5 mg/ml) was added in one well as a positive reference standard and one well filled with water as a negative control. After 24 h of incubation at 37°C, the zones of inhibition around the samples were calculated in with a graduated scale in millimeters (mm). All samples were studied in triplicate. To support these obtained data, photographs of the inhibition zones were taken and a solution of vitamin C with various concentrations was tested to check the antibacterial effect against Gram-negative and Gram-positive pathogens.

Stability of vitamin C at different temperatures and pH

Three autoclaved Eppendorf tubes were taken and (20 mg/ml) of vitamin C solution was added in each tube to evaluate the stability at various temperatures (4°C, 37°C, and 50°C). These three Eppendorf tubes were kept at (4°C, 37°C, and 50°C) for 36 h (Aramwit et al., 2010; Ramos et al., 2019). The antimicrobial action of these samples was assessed after the incubation period, by using the agar well diffusion method against Gram- negative bacteria (K. pneumoniae, E. coli, P. aeruginosa and P. mirabilis and Gram-positive bacteria (B. subtilis, B. licheniformis, and S. aureus). To record the stability of vitamin C at different pH values, three falcon tubes were taken and 20 mg/ml of vitamin C solution was added to each tube. In one tube, NaOH was added until its pH became basic or (pH 8), and in the second tube, HCl (diluted) was added dropwise till its pH became 3 or acidic. Similarly, in the third falcon tube pH was adjusted via pH meter to basic/ pH (11) by adding NaOH (Aramwit et al., 2014).

Statistical Analysis

Results were tabulated as the means ±SEM. Statistical analysis was carried out using SPSS (version 16) and the data were assessed by one-way analysis of variance (ANOVA), and Tukey’s multiple comparison test. When the P-value was 0.05, values were deliberated to be statistically substantial.

Com base no texto “**Evaluation of antibacterial activity of vitamin c against human bacterial pathogens”**, responda às questões de 1 a 5.

Questão 01 (2,0)

a) Qual o objetivo do artigo?
   Espera-se que o (a) candidato (a) consiga entender que o objetivo da pesquisa
   foi elucidar o efeito antibacteriano da vitamina C em várias temperaturas (4 ° C, 37 ° C e 50 ° C) e pH (3, 8 e 11), contra bactérias Gram-positivas e Gram-negativas em várias concentrações (5-20 mg / ml) por meio do método de difusão em ágar.

b) Qual a contribuição do resultado do estudo?

Espera-se que o (a) candidato (a) entenda que o estudo apontou que a vitamina C é um agente antibacteriano eficaz e seguro que pode ser usado no futuro como uma opção de tratamento auxiliar no combate as infecções em humanos.

Questão 02 (2,0)

De acordo com o texto, responda às seguintes questões:

a) Quais são as palavras-chave?
   Espera-se que o (a) candidato (a) compreenda que as palavras-chave são método de difusão em ágar, atividade antibacteriana, vitamina C, Pseudomonas aeruginosa.
b) 4 °C, 37 °C e 50 ° referem-se a qual informação?
   Espera-se que o (a) candidato (a) entenda que a vitamina C
   permaneceu estável nas diferentes temperaturas.

Questão 03 (2,0)

Com base no texto, o que esses estudos falam sobre a vitamina C

a) Verghese et al. (2017) –

Espera-se que o (a) candidato (a) entenda que a vitamina C é uma das alternativas baratas – que não tem efeitos adversos –, bastante disponível para o tratamento de várias infecções, incluindo o trato urinário.

b) Hong et al. (2016) –
Espera-se que o (a) candidato (a) compreenda que Hong et al. (2016) afirmaram que altas concentrações de vitamina C possuem funções imunomoduladoras e propriedades antimicrobianas que auxiliam na diminuição do risco de infecções.

Questão 04 (2,0)

De acordo com o texto, como ocorreu o processo de avaliação das atividades antibacterianas da vitamina C?

Espera-se que o (a) candidato (a) infira que as bactérias foram derramadas na placa de Petri higienizada em um fluxo de ar laminar. Depois de solidificar, as placas foram incubadas durante 24 h a 37° C. Em seguida, 50 µl de nutriente contendo organismos de teste foi adicionado às placas por meio de uma micropipeta e espalhado sobre todas as placas com uma espátula. As placas foram secas ao ar sob condições estéreis por 10 min e poços (5 mm de diâmetro) foram formados. Nos poços restantes 50 µl de solução de teste (vitamina C) com várias concentrações (5 mg, 10 mg e 20 mg / ml) foi adicionada por meio de micropipeta. Eritromicina (5 mg / ml) foi adicionada em um poço como uma referência positiva padrão e um poço cheio com água como controle negativo. Após 24 h de incubação a 37 ° C, as zonas de inibição em torno das amostras foram calculadas com uma escala graduada em milímetros (mm). Todas as amostras foram estudadas triplicadas. Para apoiar esses dados obtidos, fotografias das zonas de inibição foram tomadas e uma solução de vitamina C com várias concentrações foi testada para verificar o efeito antibacteriano contra Patógenos Gram-negativos e Gram-positivos.

Questão 05 (2,0)

Segundo o texto, como foram analisados os resultados do experimento?

Os resultados foram tabulados como as médias ± SEM. A análise estatística foi realizada no SPSS (versão 16) e os dados foram avaliados por análise de variância unilateral (ANOVA) e teste de comparação múltipla de Tukey. Quando o valor P era de 0,05, os valores foram considerados estatisticamente substanciais.