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EFFECT OF *PHYSALIS ANGULATA AH-ZE* METHANOL EXTRACT ON K2A GENE EXPRESSION IN THE *KLEBSIELLA PNEUMONIAE* ISOLATED FROM THE CLINICAL SAMPLES IN IRAQ

Kadhem Wawy Salboh*[1], Wawy Aror[2] and Saleem Marza Zarzor[3]

[1]Department of Biology- College of Science- University of AlQadyssia- AlQadysia- Iraq.
[2]Department of Biology- College of Science- University of Kufa- Najaf- Iraq.
[3]Department of Microbiology, College of Medical- University of Takrit- Takrit- Iraq.

***Corresponding Author: Kadhem Wawy Salboh**

Department of Biology- College of Science- University of AlQadyssia- AlQadysia- Iraq.

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ABSTRACT

The emergence of multidrug-resistant strains of Klebsiella poses a significant global health challenge, particularly for K. pneumoniae, which has been identified as a major concern by the World Health Organization This species harbors resistance genes bacterial resistance, including testing of 30 isolates of K. pneumoniae against tetracycline (100%) Found to be completely resistant The study examined the effect of Physalis angulata AH-ZE methanol extract concentration four concentrations (25, 50, 75, and 100 mg/mL) obtain on the regulation of the expression of the K2A gene essential for the capsule. The results showed that K2A gene expression increased at 25 mg/mL, while higher concentrations decreased expression, which was found to be completely inhibited at 100 mg/mL. Assuming concludes, the study reveals the significant effect of Physalis anggulata AH-ZE methanol on gene expression K2A gene in Klebsiella pneumoniae isolates. The findings suggest that high concentrations of the extract may inhibit K2A gene expression and Klebsiella pneumoniae isolates.

KEYWORDS: Klebsiella pneumoniae, K2A, RT-PCR, *Physalis angulate.*

INTRODUCTION

Physalis angulata, commonly known as cutleaf groundcherry, is a plant in the Solanaceae family, which is recognized for its diverse medicinal properties and traditional uses in cultures around the world.[1] Its phytochemical composition has been Extensively studied to demonstrate antimicrobial activity. anti-inflammatory and antioxidant activity.[1,2]

The rise of antibiotic-resistant bacteria has sparked renewed interest in biology, and Physalis angulata is a promising candidate for new therapeutic development.[3] In infections, Klebsiella pneumoniae de clin is relevant. with a variety of infections including bacterial infections Hematological infections[4,5] due to susceptibility to resistance to multiple antibiotics.

The key virulence factor of K. pneumoniae is the expression of specific genes, such as the K2A gene, which is associated with capsular polysaccharides to increase bacterial viability and virulence.[6] Understanding the structure of this gene is a key factor in the development of K. pneumoniae. Preventing K. pneumoniae infection is important for developing new treatment strategies. Studies have shown that Physalis angulata extract can inhibit the growth of various pathogens, including K. pneumoniae, suggesting that its pharmacokinetic properties can interfere with bacterial metabolism if altered.[7,8] Ethanol extracts from Physalis anggulata are of particular interest because they are more antimicrobial in protecting the availability of phytochemicals than any other compound.[9,10]

Research suggests that ethanol extract from Physalis angulata can modulate viral gene expression, possibly upregulate the K2A gene and reduce the pathogenicity of K. pneumoniae. Such effects may be mediated by its various mechanisms[11], including to interfering with bacterial signaling pathways or even direct interaction... There is a bacterial cell membrane. Gene expression profile There have been some changes.[12]

In vitro studies using K. pneumoniae isolates showed that Physalis angulata ethanol extract treatment resulted in significant changes in the expression levels of key virulence factors including the K2A gene.[13]

The importance of targeting infectious agents rather than simply inhibiting bacterial growth is highlighted by its ability to reduce the selective pressures that contribute to antibiotic production.[14] If natural products such as Physalis angulata is used, it can be strategies not only to limit infection but also to reduce the occurrence of resistant strains.[15]

Investigation of the effect of Physalis anggulata ethanol extract on K2A gene expression in Klebsiella pneumoniae isolated from clinical samples in Iraq could provide valuable insights into novel therapeutic strategies. Combining medical knowledge with modern scientific research can pave the way for new solutions to prevent antibiotic-induced diseases, improve patient outcomes and public health Further research is needed to identify pathways the specific mechanisms by which Physalis angulata influences bacterial gene expression are unclear.[17]

MATERIALS AND METHODS

Ethical Approval to Research

An completed ethical certificate to complete the search from the applicable committees in the Karbala University.

Bacterial isolation

Isolation was performed at Al-Hussein Hospital in Kerbala, where thirty hospitals were collected. Medical specimens were obtained from patients with suspected infection, and these specimens were transported to the laboratory under normal conditions to avoid contamination.

Upon arrival at the laboratory, samples were processed using standard microbiological techniques. Each clinical specimen was inoculated with a selection of compounds specifically designed to stimulate the growth of Klebsiella pneumoniae. The media used included MacConkey agar and CLED agar which are effective for the isolation of Gram-negative bacteria. The plates were incubated at 37 °C for 24 h, allowing bacteria to grow and form distinct colonies.

After incubation the colony is examined macroscopically. Putative colonies of Klebsiella pneumoniae were identified on the basis of a lactose solution. This resulted in pink colonies on MacConkey agar. Subsequently, biochemical tests were performed to confirm the strain isolated. These tests include oxidase, catalase, urease and fermentation tests using different carbohydrates.

Gene expression

Gene expression studies in Klebsiella pneumoniae have been greatly improved by using K2A gene-specific primers. The primers were designed to amplify the K2A gene and accurately detect its expression level. Selecting the appropriate housekeeping genes is important to ensure adequate expression. Because there is a solid reference point for comparison. General housekeeping genes include extraction of GAPDH and RNA from bacterial cultures as key steps in the process. As this allows for the extraction of high quality RNA required for subsequent analysis, the extracted RNA is spectrophotometrically checked for integrity and purity. or use gel electrophoresis Synthesis of complementary DNA (cDNA) by reverse transcription, where RNA is converted into a stable form suitable for amplification.

Quantitative PCR (qPCR) is used to measure the expression of the K2A gene, The qPCR process includes cDNA templates, primers specific for the K2A gene and housekeeping genes, and a master mix of reagents necessary for amplification Thermal cycling conditions are optimized to ensure proper amplification of the target K2A gene

Collection of plant

Physalis anggulata were collected for this study in October at local markets. Fresh plants are carefully selected based on quality and maturity. After collection, the plants were dried in a shady place to preserve their phytochemicals and avoid degradation due to direct sunlight After thorough drying, the plants were dried using a suitable plant spinner was ground into a fine powder. This powdery method simplified the extraction procedure and ensured a large surface area for subsequent extraction of bioactive compounds. The dried and powdered Physalis angulata was then stored in airtight containers to maintain integrity until further use in extraction and testing.

Etanol extraction of Physalis angulata

The production of ethanol extracts from Physalis angulata requires several systematic steps to ensure efficient extraction of bioactive compounds.

First, the pulverized plant material is carefully weighed to determine the required amount of extract. The specific concentration of ethanol is then measured, usually using a 1:10 (w/v) ratio of plant material to solvent, although this may vary depending on the desired concentration followed by plant of the powdered material together with the measured amount of ethanol in a suitable container such as a glass bottle or flask. The mixture is stirred thoroughly to completely saturate the plant material with the pigment. The container is then sealed to prevent the ethanol from decomposing and decomposing. The extraction process is carried out by incubating the mixture in a cool dark place for a specified period of time, usually 24 to 72 hours During this period the product may be shaken or stirred periodically for dispensing the mixture has worked well.

Statistical analysis

qPCR data were analyzed using the Livac method, also known as the ΔΔCt method Ct values for target genes, K2A, and housekeeping genes were recorded for each sample after qPCR was completed. ΔCt was calculated by subtracting the Ct value of the housekeeping gene

from the Ct value of the K2A gene that allowed normalization of target gene expression.

RESULTS AN DISCUSSION

The present study used RTq-PCR to assess the under the influence of various *Physalis angulata AH-ZE* methanol extract concentrations. The findings demonstrate that in isolates treated with 25 mg/mL the levels of gene expression of k2A gene were increased. While k2A gene expression decreased as the concentration increased of methanol extract.

Table 1: Gene expression of the gene (k2A) using the Livak methods in *Klebsiella pneumoniae* when using the methanol extract *Physalis angulata AH-ZE*.

Group	No. sample	CT (k2A)	CT (16srRNA)	ΔCT Test	ΔCT Control	ΔΔCT	Fold change (2^-ΔCT)	Mean	S.D.
	1	34.48	26.78	7.70	7.14	-.56	1.50		
	2	34.48	26.62	7.86	7.14	-.72	1.60		
	3	34.48	28.45	6.03	7.14	1.11	0.50		
	4	35.22	28.56	6.66	7.14	.48	0.70		
	5	34.69	25.76	8.93	7.14	-1.79	3.50	1.9	1.9±.
T1 25%	6	34.48	26.96	7.52	7.14	-.38	1.30		
	7	33.43	26.22	7.21	7.14	-.07	1.00		
	8	33.48	26.96	6.52	7.14	.62	0.70		
	9	33.6	26.33	7.27	7.14	-.13	1.10		
	10	35.70	27.33	8.37	7.14	-1.23	2.30		
	1	34.7	29.90	4.80	7.14	2.34	0.20		
	2	34.92	27.31	7.61	7.14	-.47	1.40		
	3	34.52	27.98	6.54	7.14	.60	0.70		
	4	35.61	26.18	9.43	7.14	-2.29	4.90		
	5	34.32	27.98	6.34	7.14	.80	0.60	0.7	0.7±
T2 50%	6	35.62	27.98	7.64	7.14	-.50	1.40		
	7	34.43	29.06	5.37	7.14	1.77	0.30		
	8	34.82	27.98	6.84	7.14	.30	0.80		
	9	34.12	27.98	6.14	7.14	1.00	0.50		
	10	35.02	27.50	7.52	7.14	-.38	1.30		
	1	38.71	33.05	5.66	7.14	1.48	0.40		
	2	35.71	30.78	4.93	7.14	2.21	0.20		
	3	35.56	30.77	4.79	7.14	2.35	0.20		
	4	35.71	30.78	4.93	7.14	2.21	0.20	0.4	
	5	38.41	30.60	7.81	7.14	-.67	1.60		0.4 ±
T3 75%	6	38.56	33.02	5.54	7.14	1.60	0.30		
	7	38.41	30.66	7.75	7.14	-.61	1.50		
	8	36.71	30.78	5.93	7.14	1.21	0.40		
	9	38.86	30.78	8.08	7.14	-.94	1.90		
	10	38.71	32.84	5.87	7.14	1.27	0.40		
	1	39.74	34.92	4.82	7.14	2.32	0.20		
	2	36.76	31.24	5.52	7.14	1.62	0.30		
	3	38.74	33.10	5.64	7.14	1.50	0.40		
	4	37.72	33.01	4.71	7.14	2.43	0.20		
	5	38.75	33.03	5.72	7.14	1.42	0.40	0.1	
T4 100%	6	37.75	33	4.75	7.14	2.39	0.20		).1.
	7	38.77	33.2	5.57	7.14	1.57	0.30		
	8	37.71	31.25	6.46	7.14	.68	0.60		
	9	38.73	32.94	5.79	7.14	1.35	0.40		
	10	38.93	35.9	3.03	7.14	4.11	0.10		
Control mean	C	34.43	27.29	7.14	7.14	.00	1.0	1 .0	

This level indicates there are significant differences in gene expression between the groups and compared to the control group. Graph (1) for the relative gene expression of the k2A gene showed a significant difference between the experimental treatments (T1, T2, T3, and T4) and the control group at a probability level of $p<0.05$.

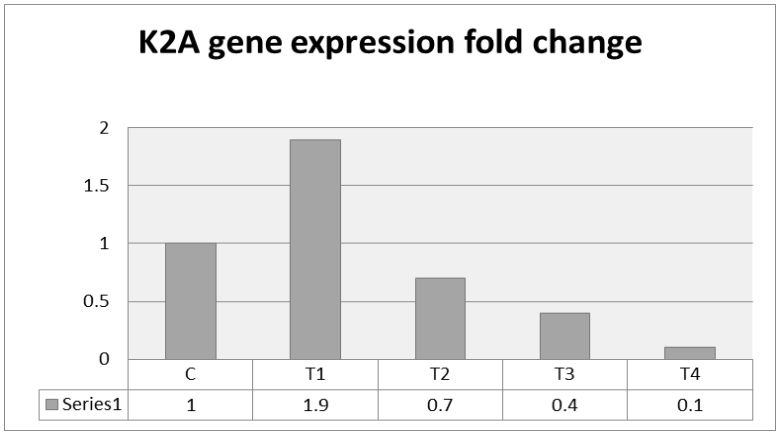

Figure 1: Rate gene expression of K2A gene in *K. pneumoniae* when was used Different concentrations of *Physalis angulata AH-ZE* methanol extract.

The concentration of 50%, 75% and 100% of the solution of the alcoholic extract of *Physalis angulata* negatively affects the level of gene expression and thus affects the formation of the biofilm of *K. pneumoniae* bacteria, which leads to a reduction in the growth of bacteria, while the concentration of 25% of the extract showed a clear effect on increasing the level of gene expression of the k2A gene which indicates the activity and effectiveness of this gene in activating and maintaining the biofilm, which leads to an increase in the growth of pathogenic bacteria. We conclude that the higher the extract concentration the lower the gene expression of the k2A gene of, indicating the efficiency of the alcoholic extract of *Physalis angulata* with a concentration of (50, 75 and 100) % as an anti-biofilm formation. These results are consistent with what was explained by the researcher (18), Some studies have indicated that about the use of the extract as an inhibitor of proteins and enzymes and that the *Physalis* extract contains some phenolic, alkaline and tannin compounds that can change the charge on the surface and then interact with the components of the cell and interact with the process of membrane formation.[19][20] Tannins inhibit enzymes and proteins present in the cell membrane, reduce the ability of the microbial cell to adhere and inhibit the transport enzymes surrounding the cell as well as the microbial and also work to form complexes with polysaccharides.[21] In conclusion, higher concentrations of the Physalis angulata extract are associated with lower expression levels of the K2A gene. This finding suggests that the alcoholic extract at concentrations of 50%, 75%, and 100% effectively inhibits biofilm formation

REFERENCES

1. Aher, T., Roy, A., & Kumar, P. Molecular detection of virulence genes associated with pathogenicity of Klebsiella spp. isolated from the respiratory tract of apparently healthy as well as sick goats. Journal of Veterinary Medicine, 2012; 67: 249–252.
2. Boonsombat, J., Chawengrum, P., Mahidol, C., Ruchirawat, S., & Thongnest, S. A new 22, 26-seco physalin steroid from Physalis angulata. Natural Product Research, 2019; 0(0): 1–8.
3. Castro-Guerrero, J. P., Ocampo-Buendia, Y. C., & Franco-Ospina, L. A. Actividad antiinflamatoria y antioxidante de Merremia umbellata (L.) Hallier f. Revista Ciencias Biomedicas, 2013; 4: 13–19.
4. Chairissy, M. D., Wulandari, L. R., & Sujuti, H. Pro-apoptotic and anti-proliferative effects of Physalis angulata leaf extract on retinoblastoma cells. International Journal of Ophthalmology, 2019; 12(9): 1402–1407.
5. Chiu, S., Wu, T., Chuang, Y., Lin, J., Fung, C., Lu, P., Wang, J., Wang, L., Siu, K., & Yeh, K. National surveillance study on carbapenem non-susceptible Klebsiella pneumoniae in Taiwan: The emergence and rapid dissemination of KPC-2 carbapenemase. PLoS ONE, 2013; 8: 1–7.
6. Chevallier, A. (2016). Encyclopedia of herbal medicine (3rd ed.). DK Publishing. Available at: http://repo.upertis.ac.id/1889/1/Encyclopedia%20Of%20Herbal%20Medicine.pdf
7. Doud, M., Zeppegno, R., Molina, E., Miller, N., Balachandar, D., Schneper, L., Poppiti, R., & Mathee, K. A K2A-positive Klebsiella pneumoniae causes liver and brain abscess in a Saint Kitt's man. International Journal of Medical Sciences, 2009; 6: 301–304.
8. Dubey, D., Raza, F., Sawhney, A., & Pandey, A. Klebsiella pneumoniae renal abscess syndrome: A rare case with metastatic involvement of lungs, eye, and brain. Case Reports in Infectious Diseases, 2013; 1–3.
9. Fang, C., Lin, Y., Lin, J., Chen, T., Yeh, K., Chang, F., Chuang, H., Wu, H., Tseng, C., & Siu, L. Klebsiella pneumoniae in the gastrointestinal tract and pyogenic liver abscess. Emerging Infectious Diseases, 2012; 18: 1322–1325.
10. Bayas-Morejon, F., Tigre-Leon, A., Tapia-Verdezoto, M., & Flores-Ribadeneira, F. Antibacterial activity of golden berry (Physalis peruviana) extract against Escherichia coli isolates from meats in Ecuador. Current Pharmaceutical Research, 2020; 12(2): 115-118.
11. Mishra, A. P., Sharifi-Rad, M., Shariati, M. A., Mabkhot, Y. N., Al-Showiman, S. S., Rauf, A.,

Salehi, B., Župunski, M., Sharifi-Rad, M., & Gusain, P. Bioactive compounds and health benefits of edible Rumex species: A review. Cellular and Molecular Biology, 2018; 64: 27-34.
12. Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences, 2018; 25: 361–366.
13. Rivieira, D. A., Ocampo, Y. C., Castro, J., Barrios, P., Diaz, L. F., & Franco, L. A. A screening of plants used in Colombian traditional medicine revealed the anti-inflammatory potential of Physalis angulata calyces, 2019.
14. Schmittgen, T. D., & Livak, K. J. Analyzing real-time PCR data by the comparative CT method. Nature Protocols, 2008; 3(6): 1101-1108.
15. Kato, S., Han, S. Y., Sun, C., Nie, X., Kang, N., Zhao, F., Chen, L., & Qiu, F. A new phenol glycoside from Physalis angulata. Natural Product Research, 2017; 6419: 1–7.
16. Turton, J., Perry, C., Elgohari, S., & Hampton, C. PCR characterization and typing of Klebsiella pneumoniae using capsular type-specific, variable number tandem repeat, and virulence gene targets. Journal of Medical Microbiology, 2010; 59: 541-547.
17. Holt, J. G., & Krieg, N. R. Bergey's Manual of Determinative Bacteriology (9th ed.). Williams & Wilkins: Baltimore, MD, USA, 1994.
18. Mishra, T., Khullar, M., & Bhatia, A. Anticancer potential of aqueous ethanol seed extract of Ziziphus mauritiana against cancer cell lines and Ehrlich ascites carcinoma. Evidence-Based Complementary and Alternative Medicine. Wiley Online Library, 2011.
19. Mishra, R., & Kaur, G. (2013). Aqueous ethanolic extract of Tinospora cordifolia as a potential candidate for differentiation-based therapy of glioblastomas. PLoS One.
20. Mishra, A., Kumar, S., Bhargava, A., et al. (2011). Studies on in vitro antioxidant and antistaphylococcal activities of some important medicinal plants. Cellular and Molecular Biology.