ORIGINAL ARTICLE

AJWA DATE (PHOENIX DACTYLIFERA) POSSESSES ACTIVITY AGAINST ESCHERICHIA COLI – AN IN VITRO STUDY

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ABSTRACT

Background: Foodborne infections are a common occurrence in the world specially since the globalization has emerged. *E. coli* is one of the main pathogens found in these infections. Different studies on different extracts of Ajwa date have pointed out its antibacterial activity, this study uses Ajwa dates as Such instead of extracts and assesses any activity against *E. coli.*

Methods: Ajwa date pulps were separated, washed, dried and 10 grams dates were blended in 100 ml water. This syrup was mixed with nutrient broth to make different concentrations of Ajwa date. *E. coli* was inoculated on agar plates to find MBC (minimum bactericidal concentration) and MIC (minimum inhibitory concentration) of Ajwa date pulp. Ampicillin 1 mg/ml was taken as standard.

Results: Ajwa date exhibited an MBC of 500 mg/ml while MIC was 250 mg/ml.

Conclusion: Ajwa date possesses both bacteriostatic and bactericidal activity against *E. coli* and can be beneficial in treating foodborne infections caused by *E. coli*.

Keywords: Ajwa date, Phoenix dactlylifera, Escheria coli, foodborne infections

How to cite this article: Rashid S, Kashif R, Naseer S, Khan N, Aisha NM**.** Ajwa Date (Phoenix Dactylifera) Possesses Activity Against Escherichia Coli – An In Vitro Study. Pak Postgrad Med J 2024;35(4): 165-168

DOI: https://doi.org/10.51642/ppmj.v35i04.594

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INTRODUCTION

Foodborne diseases are a common occurrence. Usually trivial, these may become very serious and besides economic loss, may end it mortality. According to an estimate in united states by CDC, foodborne viruses infect approximately 48 million people every year. Out of this number, almost 3000 patients die and 128,000 are hospitalized for foodborne infections.[1] Different bacteria, viruses, chemicals and parasites may contaminate food and cause these infections. Development of good and rapid trade measures across international borders has led to global economy and a wider area for spread of these diseases.[2] An outbreak of such diseases may, therefore, readily convert into huge epidemic and a potential threat to human health over a wide area.[3]

Salmonella and *Escherichia coli* are two commonly found foodborne bacteria. [4] *Escherichia coli*, usually known as *E. coli*, *belong to* gram-negative bacterial class and are usual inhabitants of food and environment. Minced beef, chicken, leafy green vegetables and sprouts are usually associated with epidemics of *E. coli.* [5] There are many strains of *E. Coli.* Most of the strains are harmless, but infection with some may result in diseases like pneumonia, respiratory infections and diarrhea.[6] Some *E. coli* also produce a toxin known as Shiga toxin. People infected with *E. coli producing* Shiga toxin exhibit diarrhea which may be bloody, stomach cramps, high grade fever, vomiting and hemolytic uremic syndrome, a potentially serious condition.[7]

Some of the current medicines used to treat this infection pose serious adverse effects that have led to explore some food substances having therapeutic potential. Date fruits (*Phenix dactylifera L.*) are among such foods. These are among staple diet in some countries and are cultivated over a large portion of the world especially Arab countries.[8] These hold a special place in Islam and Muslim culture. These are also referred to in Islamic literature and are used for various ailments and as energy boosters. [9] Ajwa dates are a variety of dates exclusively grown in Madina Munawara. These are the richest in phytochemicals among all the date fruits. It contains high amounts of fibers, amino acids, sugars, and minerals like magnesium, calcium, potassium, sodium. Vitamins as also abundant when compared to other date varieties.[10] Ajwa date flesh has abundant flavonoids, phenols and some other phytochemicals that make it a good candidate for its various therapeutic applications like anti-inflammatory, antiproliferative, antioxidant, anti-viral, anti-cancer and antifungal properties.[8] These have been tested in numerous studies. Antibacterial effects of Ajwa date have been demonstrated in many recent studies; these studies used various types of date extracts. This study has chosen Ajwa date pulp as a whole because this is the form, we usually use not the extracts.

METHODS

***Phoenx dactylifera Linn.* – Ajwa Date pulp:** Ajwa dates were procured from local date market and botanical identification was done in Botany department, Government College, Lahore. After separation from seeds, distilled water was used to rinse date pulp which was then dried with help of hot air oven. They were preserved in air tight glass containers kept at 4 °C till further use.

Escheria coli: Glycerol stock 10% was used to store *E. coli* strains kept at -80 °C. These were sub-cultured for 24 hours at 37 °C on a nutrient plate agar (Oxiod) before they were screened.[11]

Antibacterial Activity of Ajwa Date: The stored date pulps were blended in water (10 grams dates in 100 ml of water). Quantitative assessment of Ajwa date's antibacterial activity was made on Mueller-Hinton agar (Oxoid) with well diffusion technique. With a sterile cork borer, four wells (7 mm diameter) were bored, and the bacterial suspensions were inoculated on agar plates. Cell density was 1.5×10^8 colony forming units (CFU) according to McFarland standard. Test samples were filled in three wells, and a serial dilution was made with concentrations of 100 mg/ml, 200 mg/ml. 300, 400 and 500 mg/ml. Ampicillin (1 mg/ml) was used in a well taken as standard control. Ten µl ampicillin were poured in the well. These plates were kept at 37ºC and incubated for 24 hours. Inhibition zones diameter was evaluated with a vernier caliper. A comparison was made with ampicillin, the standard control. A triplicate run was performed for all tests. [12]

Determining MIC of Ajwa date pulp (Minimum Inhibitory Concentration): Ajwa dates pulp syrup was dissolved in nutrient broth and prepared in various concentrations (6.25, 125, 250, 500 and 1000 mg/mL). The density of bacterial suspension, according to McFarland standards, was 1.5 × 108 CFU. Microdilution technique was used to prepare a dilution of 1:100. Fifty µl of different Ajwa date pulp syrup concentrations were poured in a 96-well, sterile nutrient agar plate (Oxoid). Then 50 µl bacterial suspensions were added. Incubation was made for 16-20 hours at 35 °C. MIC was taken as the lowest concentration of Ajwa date pulp syrup in a well that shows no growth of bacterium (shows no turbidity).[13]

Determining MBC of Ajwa date pulp (Minimum Bactericidal Concentration): Three bacterial concentrations were prepared – one below MIC concentration, MIC concentration, and one above MIC concentration of Ajwa date pulp. MIC concentration was that concentration which showed inhibition of organism growth. Fresh oxoid nutrient plates were then sub-cultured for another incubation of 24 hours at 37 °C. MBC was the lowest Ajwa date concentration with no growth of bacterial colony on agar plates. [14]

RESULTS

Ajwa date antibacterial activity: Antibacterial activities of Ajwa date pulp were demonstrated against *E. coli*, a member of gram-negative bacterial class. The famous well-diffusion method was used for this assay. Our results showed that the lowest concentration of Ajwa date fruit pulp that inhibited growth of *E. coli* was 200 mg/mL. Increasing concentrations of Ajwa dates increased the inhibition zones.

Ajwa date MIC (Minimum Inhibitory Concentration): MIC (minimum inhibitory concentration) was used to demonstrate bacteriostatic activity of pulp of Ajwa date. Minimum Ajwa date pulp concentration at which *E. coli* growth was inhibited was 250 mg/ml

Ajwa date MBC (Minimum Bactericidal Concentration): Minimum concentration that showed absent growth of *E. coli* on nutrient agar plates taken from MIC agar plates was taken as MBC of Ajwa date fruits. A bactericidal potential of Ajwa date was shown at concentration of 250-500 mg/ml.

DISCUSSION

Foodborne infections cause some serious health problems worldwide. Their treatment with conventional antibiotics poses major adverse effects. Search for foods with

antibacterial activity is on the rise as these foods are relatively non-toxic and free from serious side effects. Ajwa dates (*Phoenix dactylifera Linn.*) are cultivated exclusively in Madina Munawwara and referred to as preventing from all ailments who eats seven of them in the morning in Hadith shareef. Scientific research on them has also proven their great potential to prevent and treat many diseases like diabetes[15], cancer [16], gastric problems[17] and many more [8]. Their antibacterial potential has also been explored and they have proven to be promising antibacterial agents.

E. coli is one of the major pathogenic bacteria found in foodborne diseases. This study has explored antibacterial potential of Ajwa date pulp as a whole against *E.* coli in an *in vitro* setting. Ajwa date when used in a concentration of 200 mg/ml inhibited the growth of *E. coli* and proved to be bactericidal when concentration reaches 500 mg/ml. From the above MIC and MBC assays, it was demonstrated that at lower concentrations, Ajwa dates exhibited bacteriostatic potential while it was bactericidal at higher concentrations. This study conforms to the results of Abdullah *et. al.,* 2019, who demonstrated antibacterial activity of Ajwa date ethanolic and methanolic extracts against various bacteria causing gastroenteritis[18].

This study shows that Ajwa dates can prevent infections caused by *E. coli* even when taken in its crude form and can be a good addition to our armamentarium against this deadly pathogen.

CONCLUSION

Study shows that Ajwa date pulp itself has antibacterial potential towards E. coli. It is bacteriostatic as well as bactericidal activities. Further study of biochemical composition of Ajwa date and its antibacterial should be carried out to find out active compounds.

CONFLICT OF INTEREST:

Authors declare no conflict of interest.

FUNDING SOURCE: None to disclose.

ETHICAL APPROVAL

Ethical approval was granted by the Ethical Committee of Sharif Medical and Dental College, Lahore, vide No SMDC/SMRC/264-22 dated: 21/01/2021

AUTHOR'S CONTRIBUTIONS

SR, SN, MMA: Conception, design, manuscript writing
RK, NK: Manuscript writing, analysis and interpretation of data,
GOQ: Data collection, critical review
ALL AUTHORS: Approval of the final version of the manuscript to be published

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