

Synergistic Antimicrobial Activity of Aqueous Extract Date Palm (*Phoenix dactylifera*) and Almond (*Prunus dulcis*) with Amoxicillin Against *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

Nawaf Raja R. Alshammari¹, Samir Qiblawi¹,
Mamoun Shadeed² and Ashfaque Hossain^{1,3*}

¹College of Medicine, University of Hail; ²College of Applied Medical Sciences, University of Hail;

³Center for Molecular Diagnostics and Personalized Therapeutics,
University of Hail, Hail, Saudi Arabia.

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Antimicrobial properties of aqueous extract of date palm and almond against Gram negative pathogens *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was investigated in this study. Synergistic antimicrobial activity between b-lactam antibiotic amoxicillin and aqueous extract of date palm and almond was assessed by disk diffusion assay and minimum inhibitory concentration (MIC) determination. Amoxicillin significantly enhanced antimicrobial activity of aqueous extract of date palm and almond against both *P. aeruginosa* and *A. baumannii*; overall synergism was more pronounced with almond extract. With aqueous extract of date palm and amoxicillin, the MIC decreased in the range of 2-4 fold for *P. aeruginosa* and 4-8 fold for *A. baumannii* strains when compared to date palm extract alone. The decrease in MIC was in the range of 8-16 fold for *P. aeruginosa* and 8-32 fold for *A. baumannii* strains when almond extract was tested with and without amoxicillin. Date palm extract with amoxicillin showed increase in zone diameter in the range of 40.6% to 89.5 % for *P. aeruginosa* and 56.8 % to 90.2 % for *A. baumannii* when compared with extract alone. Similar increase with zone diameter was noted with almond extract containing amoxicillin when compared to almond extract alone which ranged from a minimum of 56.3 % for *P. aeruginosa* to 88.5 % for *A. baumannii*. Taken together, the findings of this study show that aqueous extract of date palm and almond can act synergistically with amoxicillin against Gram negative pathogens *P. aeruginosa* and *A. baumannii*.

Keywords: *Pseudomonas aeruginosa*, *Acinetobacter baumannii*,
Date palm, Almond, MIC, Amoxicillin.

From prehistoric ages, people have been using fruits, vegetables and plant products for medicinal purpose. Use of such plant products varies with geographic locale and tradition. Infectious diseases constitute one of the major causes of death on a global scale. Various plant

products, herbs and spices from different parts of the world have been investigated as antimicrobial agents for potential treatment of infectious diseases¹. So, it is of great interest to investigate the antimicrobial nature of local fruits and plant products (date palm and almond) which are being consumed in Saudi Arabia and other Middle Eastern countries for generations.

Dates (*Phoenix dactylifera*) are an important nutritional source in many countries of

* To whom all correspondence should be addressed.
Tel.: 966-509239719
E-mail: ashfaquehossain@gmail.com

the world, because the dates contain a variety of nutrients, vitamins and minerals. Dates fruits are consumed as an indispensable component of daily diet the Middle Eastern countries for thousands of years. Various types of dates are found worldwide mainly Ajwa, Khodry, Khalas, Ruthana, Sukkary, Segae, Sefri, Munifi and Hilali and each type of date has shown medicinal value by providing health benefits. Dates and their constituents show a role in diseases prevention through anti-oxidant, anti-inflammatory, anti-bacterial activity^{2,3}. Almonds (*Prunus dulcis*) are a rich source of nutrients and phytochemicals such as vitamin E, monounsaturated fatty acids, polyunsaturated fatty acids and polyphenols⁴. Among polyphenols, flavonoids are secondary metabolites well documented for their biological effects, including anticancer, antiviral, antimutagenic, anti-inflammatory and antimicrobial activities⁵.

In this study, we analyzed aqueous extracts of date palm and almond as antimicrobial agents. As dates and almonds are being consumed in the Arabian countries from ancient ages, the rationale for this study is to determine their efficacy as antibacterial agents against two important Gram negative pathogens *P. aeruginosa* and *A. baumannii*. In addition, we also investigated synergistic activity between date palm extract and almond extract with amoxicillin, a widely used antibiotic against these two pathogens.

MATERIALS AND METHODS

Bacterial strains

P. aeruginosa and *A. baumannii* strains were obtained from King Khaled Hospital, Hail, Saudi Arabia. Trypticase soy agar (TSA) and trypticase soy broth (TSB) was used routine propagation of bacteria.

Preparation of extracts

Dates (Sukkri variety) and almond were obtained from local market and aqueous extracts were prepared as described earlier^{3,6}. Briefly, the fruits were crushed and a 20 % (w/v) aqueous suspension was made and extracted for 4 hours at room temperature with constant stirring. The suspension was then incubated at 37 °C for evaporation and the dried materials were stored at 4 °C.

Disk Diffusion Assay

Antibiotic activity of date palm and almond extracts were tested by disk diffusion method against Gram negative pathogens *P. aeruginosa* and *A. baumannii* according to Kirby-Bauer procedure described by Hudzicki⁷. Briefly, overnight cultures of test organisms were suspended in normal saline and adjusted to approximate concentration of Macfarland standard 0.5. The bacterial suspensions were then spread uniformly with sterile swab stick on Mueller Hinton agar plates. Sterile filter paper disks were then placed onto the bacterial culture thus spread on the agar plates maintaining uniform distance from each other with a sterile forcep. Different concentrations (5-20 ul) of the plant extract from a 5 % (w/v) solution were then delivered onto the filter paper disks. The plates were then kept at room temperature for 15 minutes. Then the plates were incubated at 37 °C for 24 hours. The zones of inhibitions around the disks were measured and recorded. For determining the synergistic action between date palm extract and almond extract with amoxicillin against *P. aeruginosa* and *A. baumannii*, the antibiotic at MIC strength for each organism was mixed with equal volume of the extracts immediately prior to use in the assay.

MIC determination

The Minimal Inhibitory Concentration (MIC) of an antimicrobial agent against a bacteria gives a quantitative estimate of the susceptibility of the bacteria to the test drug. Two fold serial dilutions of date palm extract and almond extracts were mixed standardized inoculums (Macfarland standard 0.5) in 200 ul final volume in microtiter plates following NCCLS⁸ guidelines. The plates were then incubated at 37 °C. The next day, the MIC was recorded as the lowest concentration of the extracts with no visible growth in the microtiter well. For determining the modulatory effect of amoxicillin on inhibition of growth of bacteria by the extracts, the antibiotic at MIC strength for each organism was mixed with equal volume of the extracts immediately prior to assay.

RESULTS AND DISCUSSION

Aqueous extracts of date palm and almond showed antimicrobial activity against clinical isolates *P. aeruginosa* and *A. baumannii*.

Table 1. Fold decrease in MIC of aqueous extract of date palm and almond against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in presence of amoxicillin

Bacterial strain	Clinical source	Date palm extract	Almond extract
AB-1	Blood	4	16
AB-2	Wound	4	32
AB-3	UTI	8	8
PA-W2	Wound	2	8
PA-W6	Wound	4	8
PA-W14	Wound	4	16

We used water extracts as dates and almonds are exposed to body fluids during the process of digestion, absorption and metabolism. So, we reasoned that analysis of aqueous extract instead of organic solvent extracts will generate more physiologically relevant data. The assays used in this study, namely disk diffusion and MIC determinations are extensively used techniques in antibiotic research.

The bacterial strains used in this study along with their clinical source are listed in the Table 1. These are well characterized strain previously used by our group^{9,10}. Date palm and almond extract produced zones of inhibition

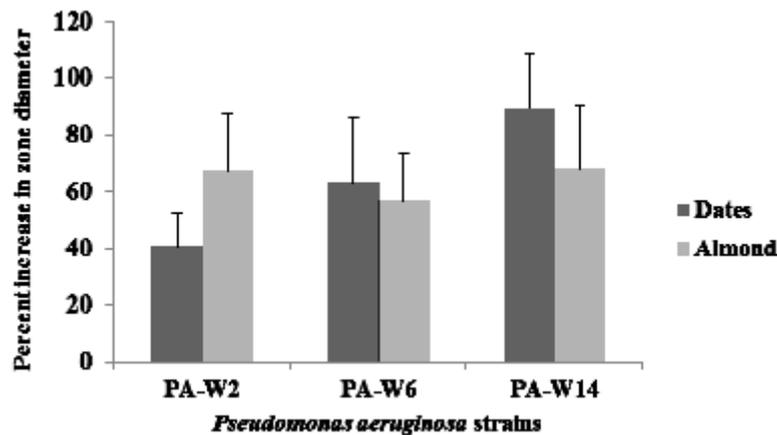


Fig. 1. Percent increase in synergistic zone of inhibition diameter of date palm and almond aqueous extracts and amoxicillin against clinical isolates of *P. aeruginosa*. Experimental procedure is described in the methods and materials section. The results are expressed as mean plus standard deviations of three independent experiments

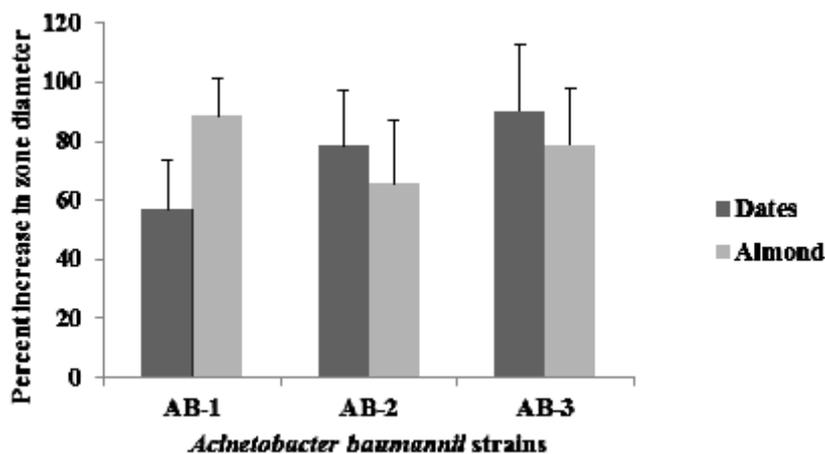


Fig. 2. Percent increase in synergistic zone of inhibition diameter of date palm and almond aqueous extracts and amoxicillin against clinical isolates of *A. baumannii*. Experimental procedure is described in the methods and materials section. The results are expressed as mean plus standard deviations of three independent experiments

against *P. aeruginosa* and *A. baumannii*. Saleh and Otaibi¹¹ analyzed antibacterial activity of date palm in different stages of ripening against and reported enhanced activity against Gram positive pathogens in comparison to the Gram negative pathogens. Aamir *et al*¹² reported that methanol extract of date seed exhibited synergistic antimicrobial activity with methanol extract of seed of medicinal plant *Annona Squamosa* against different Gram positive and Gram negative bacterial species. However, synergistic antimicrobial of date palm extract / almond extract and amoxicillin against Gram negative pathogens was not evaluated before.

To assess synergistic antimicrobial activity of aqueous extracts with amoxicillin, we determined the zones of inhibition of date palm and almond extracts alone and in combination with amoxicillin. We then calculated the percent increase zone diameter of the extracts in presence amoxicillin and considered that as an index of synergism between the extracts amoxicillin. The Percent increase in synergistic zone of inhibition diameter of date palm and almond aqueous extracts with amoxicillin against clinical isolates of *P. aeruginosa* and *A. baumannii* are presented in Fig. 1 and Fig. 2, respectively. Comparison of susceptibility between the organisms *P. aeruginosa* and *A. baumannii* to the synergistic action of date palm / almond extract and amoxicillin showed that almond extract : amoxicillin combination exhibited stronger synergistic effect than the date palm extract and amoxicillin combination (Fig. 1 and Fig. 2).

We determined MIC of aqueous extracts of date palm and almond both in presence and absence of amoxicillin to determine whether the antibiotic exhibits any synergistic activity along with these extracts in this assay. We expressed the synergistic effect as fold decrease in MIC when date palm / almond extract : amoxicillin combination was used in comparison to the MIC of the date palm / almond extracts alone (Table 1). Pronounced decrease in MIC value; 2-8 fold for date palm extract and 8-16 fold for almond extract in presence of amoxicillin extract implied strong synergistic activity between antibiotic and the extracts. In comparison to date palm extract, almond extract exhibited stronger synergistic activity with amoxicillin which is exemplified by 8-16 fold decrease in MIC of amoxicillin-date palm/ almond

extract. In general, *A. baumannii* strains exhibited enhanced sensitivity to date palm / almond extract : amoxicillin combination in comparison to the *P. aeruginosa* strains. Overall, no correlation was observed between the clinical source of the strain and the synergistic action. Studies with larger number of strain will help generate a clear picture in this regard.

In conclusion, our preliminary investigation showed that the aqueous extracts of date palm and almond exhibited synergistic antimicrobial activity with amoxicillin against Gram negative pathogens *P. aeruginosa* and *A. baumannii*. The important finding that stems from this study is that concomitant intake of dates or almond and the antibiotic amoxicillin may not impair the activity of the drug. Experiments are in progress in our laboratory to determine the spectrum of synergism exhibited by date palm / almond extracts with a panel of antibiotics against different Gram positive and Gram negative microorganisms.

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