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Antibacterial and antibiofilm activity of Mexican Mejhoul date (*Phoenix dactylifera* L.) seed extract against pathogenic bacteria

Actividad antibacteriana y antibiopelícula del extracto de semilla de dátil mexicano Mejhoul (*Phoenix dactylifera* L.) contra bacterias patógenas

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ABSTRACT

The incidence of infections in hospital environments is a critical global health issue, compromising people's health and lives. The current paradigm of disinfection focuses on the resistance of bacteria growing as biofilms, which gives antibiotic resistance. Natural antibacterials from fruit byproducts are alternative solutions to reduce the use of antibiotics and synthetic disinfectants. This study aimed to explore the antibacterial and antibiofilm activity

of date seed extract against pathogenic bacteria. Characterization of date seed extract revealed a total phenolic and flavonoid content of 34.67 ± 1.77 mg GAE/g and 123.06 ± 2.94 mg QE/g, respectively; gallic and cinnamic acid were also identified in the extract. Minimum inhibitory and bactericidal concentrations were 12 and 16 mg/mL, for *Escherichia coli* and *Pseudomonas aeruginosa*; while for *Staphylococcus aureus* were 8 and 12 mg/mL, respectively. A sub-inhibitory concentration (0.5 MIC) inhibited biofilm formation of the pathogens, reducing the viable cells adhered to stainless steel surfaces at 30 min by 1.03, 0.16, and 1.62 log CFU/cm^2 and at 24 h of incubation by 0.26, 2.07, and 4.15 log CFU/cm^2 for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively. These results demonstrate the potential of mexican Mejhoul date seed as a source of bioactive compounds with antibacterial and antibiofilm activity.

Keywords: biofilm, byproducts, Mejhoul, nosocomial pathogens, phenolic compounds, surface contamination.

RESUMEN

La incidencia de infecciones en entornos hospitalarios es un problema crítico de salud global, que compromete la salud y la vida de las personas. El paradigma actual de desinfección se centra en la resistencia de las bacterias que crecen como biopelículas, lo que genera resistencia a los antibióticos. Los antibacterianos naturales provenientes de subproductos de frutas son soluciones alternativas para reducir el uso de antibióticos y desinfectantes sintéticos. Este estudio evaluó la actividad antibacteriana y antibiopelícula del extracto de semilla de dátil frente a bacterias patógenas. El extracto mostró un contenido de fenoles y flavonoides de 34.67 ± 1.77 mg EAG/g y 123.06 ± 2.94 mg EQ/g, respectivamente, con presencia de ácido gálico y cinámico. Las concentraciones mínimas inhibitorias y bactericidas fueron 12 y 16 mg/mL para *Escherichia coli* y *Pseudomonas aeruginosa*, mientras que para *Staphylococcus aureus* fueron 8 y 12 mg/mL. Además, una concentración subinhibitoria (0.5 CMI) redujo 1.03, 0.16 y 1.62 log UFC/cm^2 las células viables adheridas a superficies de acero inoxidable a los 30 min y 0.26, 2.07 y 4.15 log UFC/cm^2 a las 24 h de incubación para *E. coli*, *P. aeruginosa* y *S. aureus*, respectivamente. Estos resultados evidencian el potencial de la semilla de dátil Mejhoul mexicano como fuente de compuestos bioactivos con actividad antibacteriana y antibiopelícula.

Palabras clave: biopelículas, compuestos fenólicos, contaminación de superficies, Mejhoul, patógenos nosocomiales, subproductos.

1. INTRODUCTION

Bacterial infections are a major global health concern, contributing significantly to morbidity and mortality worldwide. Infections, such as those of the urinary tract, lungs, skin, soft tissues, among others, can originate from various environments, including healthcare settings, representing a persistent risk and significant impact on health. The transmission of pathogens is complex and can occur from person to person through direct contact, shared elements, or cross-contamination between surfaces (Monegro *et al.,* 2017). A single-hand

contact with a contaminated surface results in a variable degree of pathogen transfer (Pegu *et al.,* 2021).

The contamination of surfaces with pathogenic bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, among others, plays an important role in the incidence of infections. These pathogens can survive on dry, inanimate surfaces for hours to several months, depending on factors such as the surface, humidity, and temperature conditions, even after surface disinfection (Porter *et al.,* 2024). This persistence is attributed to their ability to form biofilms, bacterial aggregates embedded in an extracellular polymeric matrix. Biofilms can be up to 1000 times more resistant to antimicrobials than individual bacteria (Prinzi and Rohde 2023). Although there are surface decontamination protocols, the continued use and high doses of disinfectants and antibiotics can become toxic and cause bacterial resistance (Rozman *et al.,* 2021). Therefore, research has focused on using more natural alternatives, such as plant extracts. It has been reported that plant extracts, such as those from agricultural byproducts, are important sources of molecules with a broad spectrum of antimicrobial action (Bernal-Mercado *et al.,* 2018, Gettens *et al.,* 2021, Mejía-Barajas, 2020). Comprehensive utilization of agro-industrial byproducts is essential in addressing environmental sustainability and resource efficiency.

The fruit from date palm (*Phoenix dactylifera* L.) showed a high profile of bioactive compounds to which a medicinal role in treating various diseases has been attributed. The date seed represents 10-15% of the fruit's weight and is the industry's main byproduct (Golshan Tafti *et al.,* 2017). Several studies have reported significant phenolic compounds such as flavonoids and phenolic acids in date pulp and seed (Salomón-Torres *et al.,* 2019, Osamede Airouyuwa *et al.,* 2022). For example, the date seeds from Majul, Khalas, and Zahedi cultivars showed a total phenolic content between 14.46-35.41 mg of gallic acid equivalents per gram (mg GAE/g) (Ardekani *et al.,* 2010, Abuelgassim *et al.,* 2020, Shi *et al.,* 2023). Similarly, Salomón-Torres *et al.,* (2019) reported a phenolic content of 13.73 mg GAE/100 g in mexican Mejhoul date seeds. Phenolic compounds have demonstrated antimicrobial and antibiofilm activity against different pathogenic bacteria (Vazquez-Armenta *et al.,* 2018, Bernal-Mercado *et al.,* 2020). Therefore, harnessing the bioactive compounds in date seeds will add value to these byproducts and contribute to developing antimicrobial and antibiofilm agents. In this sense, this study aimed to evaluate the potential of mexican Mejhoul date seed extract to inhibit pathogenic bacteria's growth and biofilm formation.

2. MATERIALS AND METHODS

2.1. Plant material

Date seeds were obtained from Mejhoul cultivar (in the Tamar stage of maturity), which was cultivated in 2021 in San Luis Rio Colorado Valley in Sonora, Mexico, and stored at -20 °C before the extraction.

2.2. Preparation of date seed extract

The date seed was crushed to obtain a powder, and 10 g was placed in 100 mL of ethanol: water (7:3 v/v) and left in darkness for 10 days at 25 °C. The extract was filtered, and the ethanol was removed using a rotary evaporator (Yamato Scientific Co., RE301 series) at reduced pressure and 45 °C. The evaporation residue was subjected to alkaline hydrolysis (10 mL of NaOH 4 M) for four hours without light, followed by acid hydrolysis with HCl 4 M until reaching a pH of 2. Finally, the extracts were frozen at -30 °C and lyophilized to obtain them in powder form for subsequent determinations (Ayala-Zavala *et al.,* 2012).

2.3. Total phenolic content

The experiment was based on the Folin-Ciocalteau methodology with slight modifications (Singleton and Rossi 1965). For the assay, 50 µL of the sample (2.5 mg/mL) dissolved in 3 mL of distilled water was mixed with 250 µL of 1 N Folin-Ciocalteau reagent (Sigma-Aldrich) and left to rest for 5-8 min. Subsequently, 750 µL of 20% Na_2CO_3 and 950 µL of distilled water were added. The mixture was shaken vigorously and left to rest for 30 min. Absorbance at 765 nm was measured using a FLUOstar Omega spectrophotometer (BMG Labtech Inc., Model Omega, Chicago, IL, USA). Total phenolic compounds were calculated using a standard curve obtained from known dilutions of gallic acid. The results were expressed as mg of gallic acid equivalents per gram of dry weight of the extract (mg GAE/g d.w.) (Ayala-Zavala *et al.,* 2012).

2.4. Total flavonoid content

The determination of total flavonoids was performed using the method described by Zhishen *et al.,* (1999) with some modifications. A mixture of 100 µL of the sample (2.5 mg/mL) and 430 µL of mixture A (1.8 mL of 5% $NaNO_2$ with 24 mL of distilled water) was developed and allowed to settle for 5 min. Subsequently, 30 µL of 10% $AlCl_3$ was added and left for another minute. Then, 440 µL of mixture B (12 mL of 1 M NaOH with 14.4 mL of distilled water) was added. The absorbance of the mixtures was measured after the reaction at 496 nm in a FLUOstar Omega spectrophotometer (BMG Labtech Inc., Model Omega, Chicago, IL, USA). For this case, a calibration curve with quercetin was used, and the results were expressed as mg of quercetin equivalents per gram of extract in dry weight (mg QE/g d.w.).

2.5. Chromatographic identification of phenolic compounds

The extracted phenolic compounds of DSE were identified and quantified in a diode array detector ultra-performance liquid chromatography system (UPLC-DAD; ACQUITY, Waters Corp., Milford, MA, USA), as previously reported by Velderrain-Rodríguez *et al.,* (2018). Separation was performed on a BEH C18 column (3.0 mm × 100 mm, 5 µm, Waters) at 60 °C, and the mobile phases were 0.5% formic acid (A) and methanol (B). A gradient elution was applied as follows: 0-0.25 min (80% A-20% B, 0.4-0.15 mL/min), 0.25-5 min (80% A-20% B, 0.15-0.2 mL/min), 5-12 min (80-55% A- 20-45%B, 0.2-0.18 mL/min), 12-25 min (55-0% A- 45-100 %B, 0.18-0.10 mL/min), 25-26 min (0-60 %A- 100-40% B, 0.1-0.2 mL/min), 26-27 min (60-80 %A- 40-20% B, 0.2-0.4 mL/min), 27-30 min (80% A-20% B, 0.4 mL/min). The eluted compounds were identified at 280 nm by comparing their retention

times and absorption spectra with gallic and cinnamic acid commercial standards (Sigma-Aldrich). They were quantified using standard curves prepared with the same standards.

2.6. Minimal inhibitory and bactericidal concentration

The antibacterial capacity of DSE against bacteria from nosocomial environments, uropathogenic *Escherichia coli* ATCC 70016, *Pseudomonas aeruginosa* ATCC 10154, and *Staphylococcus aureus* ATCC 6538, were determined. The inoculum was prepared using an 18 h culture adjusted reading the absorbance at 600 nm using a microplate reader (FLUOstar Omega, BMG Labtech, Chicago, IL, USA) to achieve a final concentration of 1×10^6 colony forming units per milliliter (CFU/mL). The antibacterial effectiveness of the extract was determined using the broth microdilution method, for which different dilutions of the extract were prepared in Mueller Hinton (MH) broth (Difco™) in a range of 0 to 30 mg/mL every 2 mg/mL. Five microliters of the inoculum and 295 µL of the extract dilutions were added to 96-well polystyrene microplates (Costar 96) and incubated for 24 h at 37 °C. The lowest extract concentration that inhibits the visible bacterial growth (absence of turbidity) was determined as the Minimal inhibitory (MIC). Samples (20 µL) from tubes without visible growth were plated in MH agar, and the lowest concentration without viability was considered the Minimal bactericidal concentration (MBC) (Burt *et al.,* 2005). The assay was performed by triplicate.

2.7. Inhibition of total biofilm biomass

The effect of DSE on biofilm formation was also followed by using the crystal violet assay to measure the total biomass produced by bacteria (Tapia-Rodriguez *et al.,* 2023). Bacterial inoculum was prepared to achieve a final 1×10^6 CFU/mL concentration in MH broth from an exponential phase culture (18 h in MH broth). For the assay, 5 µL of the bacterial solution and 295 µL of non-inhibitory concentrations (0.25 and 0.5 MIC) of DSE in MH broth were placed in sterile 96-well polystyrene microplates (Costar 96). Then, the microplate was incubated for 24 h at 37 °C. After that, the medium was removed by aspiration, washed three times with distilled water, and dried for 15 min.

Afterward, at room temperature, biofilms were stained with 150 µL of 0.1% (w/v) crystal violet for 45 min. The unbound dye was removed by gently washing the wells three times with distilled water. The microplates were dried for 15 min, and each well's crystal violet was solubilized by adding 150 µL of 33% acetic acid for 15 min. Then, the solubilized dye of each well was transferred to another microplate, and the OD was measured at 600 nm in a FLUOstar Omega spectrophotometer (BMG Labtech, Chicago, IL, USA). Each measure was performed in triplicate, and MH broth (without any treatment) was used as a blank. Results were expressed as the percentage of reduced biomass produced (OD), considering 100% the value of the control bacterial biomass.

2.8. Effect of date seed extract on bacterial adhesion

The effect of DSE on the biofilm formation of *E. coli*, *P. aeruginosa*, and *S. aureus* was evaluated by measuring viable cells attached to stainless steel surfaces (Vazquez-Armenta *et al*., 2017), a common surface material in a nosocomial environment. For the test, 6 mL of MH broth containing the extract and stainless steel coupons (1 × 1 × 0.1 cm) were inoculated

with $1x10^6$ CFU/mL of each bacterium and incubated at 37 °C for 24 h. Subsequently, coupons were removed at 30 min and 24 h, washed with sterile saline solution (2 mL) to remove weakly adhered cells, and sonicated (40 kHz) for 5 min in 3 mL of sterile saline solution. Serial dilutions were made and then cultured in MH agar. The assay was performed in triplicate, and the results were expressed as a logarithm of colony-forming units of viable adhered cells per square centimeter (log CFU/cm^2).

2.9. Statistical analysis

A completely randomized experimental design was carried out for all experiments. For the characterization of the extract, the data were expressed as the mean of three replicates ± standard deviation. For the biofilm formation inhibition, the factor was the concentration of the extract, and the response variables were the number of adhered cells (log CFU/cm^2) and biomass reduction (%). An ANOVA ($p \leq 0.05$) was performed to estimate significant differences between the treatment and the control, and the mean comparison test was applied by the Tukey-Kramer method. The statistical software NCSS 2022 was used to analyze the results.

3. RESULTS

3.1. Total phenolic and flavonoid content

The total phenolic and flavonoid content was evaluated in DSE, which showed a higher content of flavonoids (123.06 ± 2.94 mg QE/g d.w.) than total phenolics (34.67 ± 1.77 mg GAE/g d.w.). Additionally, DSE was characterized using UPLC-DAD being gallic and cinnamic acids the compounds identified in the seed extract (Fig. 1). Besides, the quantification using standard curves revealed that gallic acid was in higher concentration in DSE (5.13 ± 0.30 mg/mL) than cinnamic acid (1.00 ± 0.03 mg/mL).

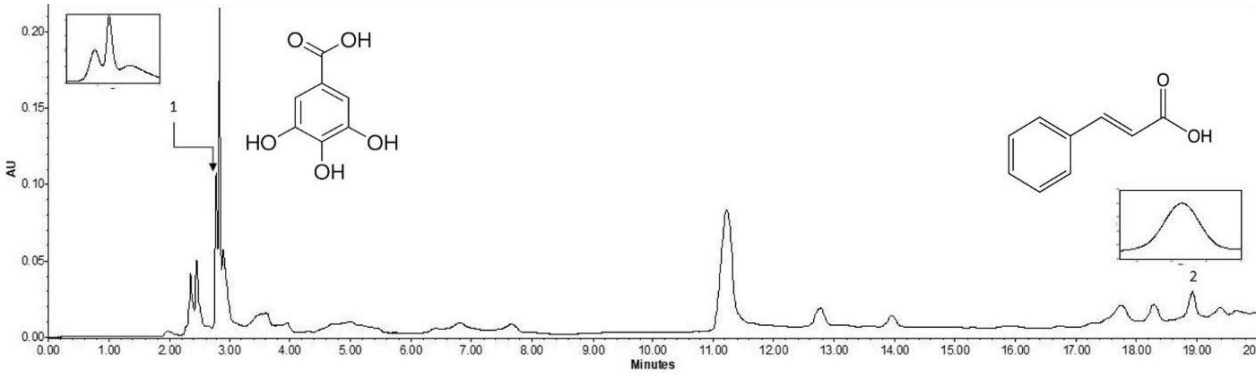

Fig. 1. Representative UPLC-DAD chromatogram used to identify and quantify the phenolic compounds in DSE. 1) gallic acid, 2) cinnamic acid.

3.2. Antibacterial activity

The broth microdilution method was used to evaluate the inhibitory activity of DSE against the nosocomial pathogens *E. coli*, *P. aeruginosa*, and *S. aureus*. DSE effectively inhibited the growth of all three tested pathogens (Table 1). The MIC required to suppress the bacteria growth was in the range of 8-12 mg/mL, whereas the concentration of 12-16 mg/mL was required to achieve a bactericidal effect. These results indicate that *S. aureus* was the most susceptible bacteria to DSE, as it exhibited lower MIC and MBC values compared to the Gram-negative bacteria. The similar response observed in *E. coli* and *P. aeruginosa* suggests a comparable degree of resistance to the extract among these Gram-negative strains.

Table 1. Minimal inhibitory and bactericidal concentration of date seed extract against pathogenic bacteria.

Bacteria	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli UPEC	12	16
Pseudomonas aeruginosa	12	16
Staphylococcus aureus	8	12

3.3. Antibiofilm activity

The effect of DSE (at a non-inhibitory concentration) on the biofilm formation process of pathogenic bacteria was evaluated as the total biofilm biomass and the number of viable cells adhered to stainless steel surfaces. It can be observed that DSE significantly reduced ($p \leq 0.05$) biofilm biomass at 0.5 MIC, showing inhibition values of 59.6, 29.4 and 9.9% for *E. coli*¸ *P. aeruginosa* and *S. aureus,* respectively, whereas for 1 MIC were in the range of 49.3-74.2% (Fig. 2), being only *E. coli* biofilms susceptible to 0.25 MIC. Considering the effect of 1 MIC on bacterial growth, the 0.5 MIC concentration was used in the bacterial adhesion assay. DSE reduced ($p \leq 0.05$) the number of viable cells attached to stainless steel surfaces after 30 min of incubation at 37 °C (Fig. 3), with a reduction of 1.03, 0.17, and 1.62 log CFU/cm^2 for *E. coli*, *P. aeruginosa*, and *S. aureus*, respectively, compared to the controls. Additionally, during the 24 h of incubation, the inhibitory effect of the extract was maintained, obtaining reductions in cell adhesion of 0.26, 2.07, and 4.15 log CFU/cm^2 for *E. coli*, *P. aeruginosa,* and *S. aureus*, respectively (Fig. 4). At the end of the incubation period, it was observed that *S. aureus* biofilms were the most affected by DSE.

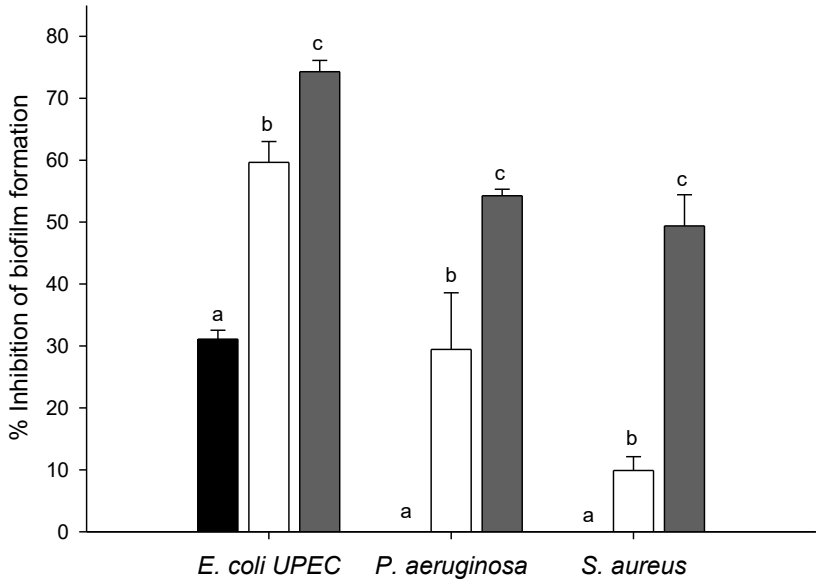

Fig. 2. Percentage of inhibition of *E. coli*, *P. aeruginosa*, and *S. aureus* biofilms incubated at 37 °C for 24 h in the presence of sub-MIC's DSE concentrations: 0.25 MIC (black bars), 0.5 MIC (empty bars), 1 MIC (gray bars), determined by crystal violet assay. Values are means ± standard deviation, n= 3. Different literals among concentrations indicate significant differences, p≤0.05.

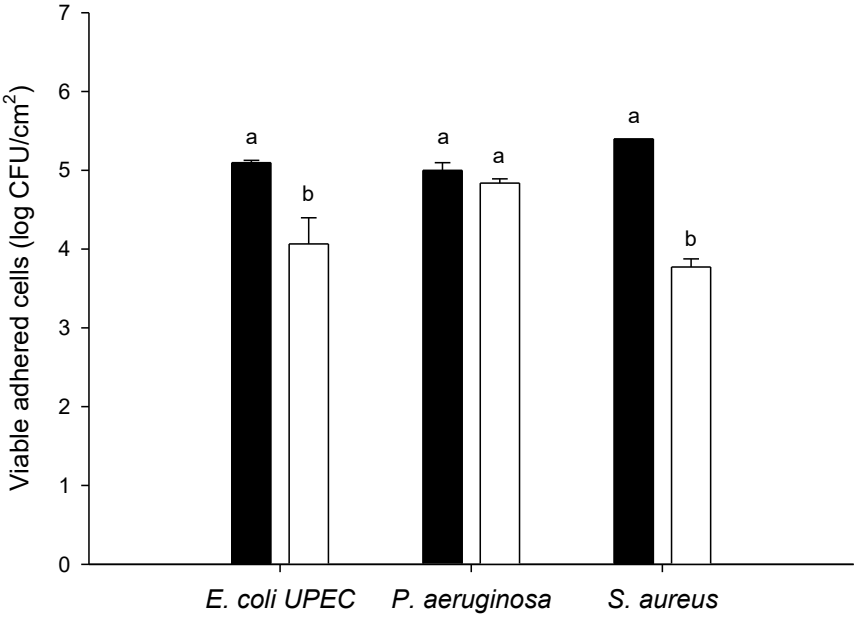

Fig. 3. Effect of DSE at 0.5 MIC (empty bars) on initial cell density of biofilms adhered to stainless steel surfaces incubated at 37 °C for 30 min, compared to control (black bars).

Values are means ± standard deviation, n= 3. Different literals indicate significant differences, p≤0.05.

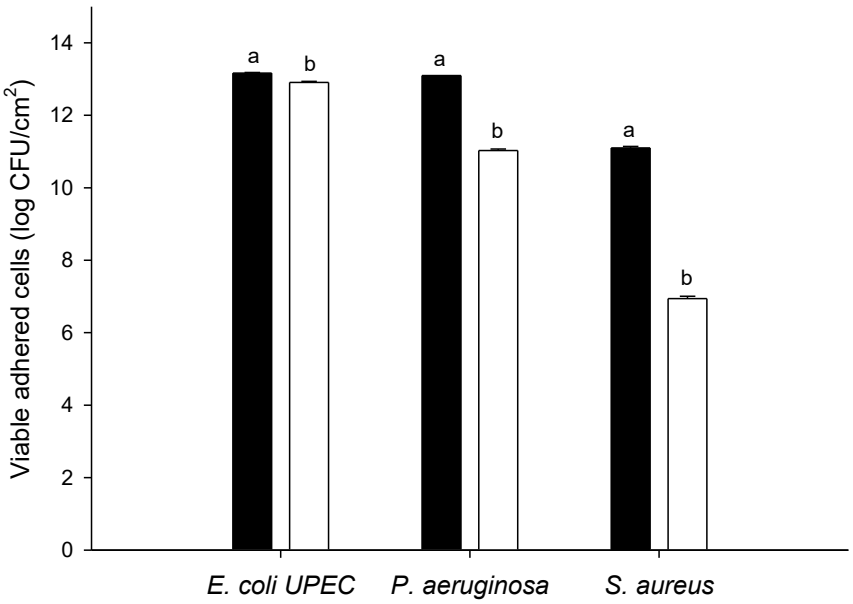

Fig. 4. Effect of DSE at 0.5 MIC (empty bars) on cell density of mature biofilms adhered to stainless steel surfaces incubated at 37 °C for 24 h, compared to control (black bars). Values are means ± standard deviation, n= 3. Different literals indicate significant differences, p≤0.05.

4. DISCUSSION

Phenolic compounds are phytochemicals widely found throughout various plant materials, such as fruits, and are secondary metabolites generated via shikimic acid and phenylpropanoid pathways. The findings of our study reveal a significant phenolic and flavonoid content in DSE from the Mejhoul dates cultivated in the San Luis Río Colorado Valley. These results are particularly noteworthy, as they highlight the unique environmental conditions of the region, such as arid climate, high temperatures, and limited rainfall during the harvest season, which have been associated with increased production of secondary metabolites in plants (Salomon-Torres et al., 2019). The phenolic content of 34.67 mg GAE/g reported in this study is comparable to previous reports. For instance, Ardekani *et al.,* (2010) reported a total phenolic content of 35.41 mg GAE/g for the Zahedi cultivar, while other cultivars such as Zahedi, Kabkab, Mazafati, and Rabbi showed a range of 14.80-33.80 mg GAE/g (Radfar *et al.,* 2019). Our study's phenolic content is significantly higher than the 14.46-18.53 mg GAE/g reported by Shi *et al.,* (2023) for Mejhoul cultivar using conventional and ultrasonic-assisted extraction methods. Additionally, our results are higher than the reported by Abuelgassim *et al.,* (2020) for the Sukkari and Khalas cultivars. Regarding total flavonoids, DSE contains 123.06 mg QE/g d.w., which is significantly higher compared to

the 0.83-0.94 mg QE/g reported by Abuelgassim *et al.,* (2020) and aligns closely with the 35.28 mg QE/g d.w. reported by Anwar *et al.,* (2022) for the Awjar cultivar.
Furthermore, Ghafoor *et al.,* (2022) reported a total phenolic and flavonoid content for Mejhoul cultivar obtained by soxhlet, subcritical CO_2 extraction, and supercritical fluid extraction methods, with values of 99.13, 274.98, and 171.44 mg GAE/100 g for phenolic content and 47.30, 123.23, and 90.04 mg QE for flavonoids, respectively. The findings of our study underscore the importance of the San Luis Rio Colorado Valley's unique environmental conditions mentioned above, which contribute to the higher phenolic and flavonoid contents observed in the Mejhoul date seeds, thereby enhancing their potential as a source of bioactive compounds with significant antibacterial and antibiofilm activities.
The higher content of flavonoids in the Mejhoul cultivar coincides with those reported in previous studies, in which the presence of flavonoid glycosides of luteolin, quercetin, and apigenin was observed in dates of the Deglet Noor cultivar (Hong *et al.,* 2006). The predominant bioactive compounds reported in date seeds include the phenolic acids, gallic, *p*-hydroxybenzoic, protocatechuic, *m*-coumaric, caffeic, and ferulic acids, as well as the flavonoids epicatechin, catechin, and rutin (Maqsood *et al.,* 2015). In our study, only gallic and cinnamic acids were identified in the extract. This result concurs with those Bouhlali *et al*. (2018) reported, which also identified gallic acid in a methanolic DSE from Mejhoul cultivar. Also, other authors reported the presence of the same compounds, such as Al Harthi *et al.,* (2015), who reported the presence of gallic acid in date seed ethanolic extracts of Faradh, Khasab, Bunarinja, and Khasab cultivars from Oman. On the other hand, gallic and cinnamic acid derivatives have been reported in methanolic extracts from Aseel, Karbalaen, and Khupro cultivars (Majid *et al.,* 2023). All studies reported the presence of other compounds in addition to gallic and cinnamic acids. Among the factors that affect the variability in the content of phenolic compounds in the date seed are the cultivar, the geographical region, and the climatic conditions (Allaith, 2019). Delineating dates' composition, cultivar, ripening stage, and bioactive fractions are important when designing and interpreting research studies. For consistent compositional reporting, standardization of extraction and analytical methods is needed (Al-Dashti *et al.,* 2021).

These compounds, particularly phenolic acids and flavonoids, are known for their bioactive properties, including antibacterial and antibiofilm activities. In our study, the DSE exhibited significant antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*. The different susceptibilities of bacteria to DSE can be attributed to their structural differences because *S. aureus*, the Gram-positive bacteria, was more sensitive to the extract. In contrast, the Gram-negative bacteria, *E. coli,* and *P. aeruginosa*, were more resistant. A thick layer of peptidoglycan surrounds the cytoplasm of Gram-positive bacteria, while in Gram-negative bacteria, an additional outer membrane containing mainly lipopolysaccharides is present (Fattouch *et al.,* 2007). It has been reported that the antibacterial effect of phenolic compounds against Gram-positive bacteria is associated with their ability to interact directly with the peptidoglycan layer, affecting cell integrity and increasing bacterial sensitivity to osmotic pressure and ionic strength. However, the lipopolysaccharide layer on the outer membrane of Gram-negative bacteria functions as a permeability barrier that prevents the interaction of phenolic compounds with the peptidoglycan layer (Papuc *et al.,* 2017). As previously mentioned, the date seed has phenolic compounds such as phenolic acids and flavonoids, among others, to which antimicrobial properties have been attributed. It has been hypothesized that the mechanism of action of some phenolic compounds is attributed to

their capacity to internalize into bacterial membranes, causing a destabilization, interaction with DNA and proteins, inhibition of nucleic acid synthesis, and a major synthesis of intracellular reactive oxygen species (Shi *et al.,* 2021, Zhang *et al.,* 2022).

The inhibitory activity DSE has also been observed in other cultivars such as Halawi, Khadrawi, and Zahdi, whose ethanolic extracts inhibited the growth of various pathogenic bacteria, including *E. coli*, *P. aeruginosa* and *S. aureus* (Aljazy *et al.,* 2019). Radfar *et al.* (2019) reported that the MIC and MBC of DSE of Zahedi, Kabkab, Mazafati, and Rabbi cultivars against *S. aureus* were in the range of 1.56-3.125 and 3.125-12.5 mg/mL, respectively. The differences between the DSE from Mejhoul cultivar and the studies mentioned above can be attributed to the different cultivars used and the extraction methods. However, it is important to emphasize that the environmental conditions and cultivation practices in the San Luis Rio Colorado Valley contribute significantly to the enhanced antibacterial activity observed in our study.

Furthermore, the biofilm inhibitory effects of DSE were pronounced, particularly against *P. aeruginosa*, suggesting its potential as a biofilm-disrupting agent. This is consistent with other studies reporting the efficacy of DSE in biofilm inhibition such as that of Al-Tamimi *et al.,* (2021), whose reported that methanol-hexane seed extracts from Ajwa and Safawi dates also inhibited *P. aeruginosa* and *S. aureus* biofilms, with inhibition levels below 20% at sub-MIC doses. Similarly, ethanolic extracts from El Wadi cultivar caused an inhibition of *E. coli* and *S. aureus* biofilms by 74.46 and 98.59%, respectively (Gomaa *et al.,* 2024).
Additionally, our study observed that DSE from the Mejhoul cultivar reduced the number of viable cells attached after 30 min and maintained the inhibitory effect at 24 hours of incubation. High biofilm reductions were observed in *P. aeruginosa* and *S. aureus*. Another study demonstrated that polyphenol extracts of Ajwa cultivar inhibited biofilm formation in 75% of *S. aureus*, 60% of *P. aeruginosa*, 80% of *Salmonella enteritidis*, and 50% of *E. coli* at concentrations of 40.50 µg/mL, 90.75 µg/mL, 80.40 µg/mL, and >100 µg/mL respectively. Safawi cultivar extracts exhibited biofilm inhibition percentages of 65.78% for *S. aureus*, 45.5% for *P. aeruginosa*, 54.19% for *S. enteritidis*, and 54.19% for *E. coli* at concentrations of 65.50 µg/mL, 88.50 µg/mL, 98.50 µg/mL, and >100 µg/mL, respectively (Al-Tamimi, Alfarhan, & Rajagopal, 2021).

The differences observed between bacteria can be attributed to the different mechanisms of biofilm inhibition of DSE compounds. Several studies have reported that phenolic compounds can inhibit biofilm formation by affecting motility, secretion of polymeric substances, or even altering or stopping the intercellular communication systems (Ong *et al.,* 2018, Kauffmann and Castro, 2023). For example, gallic acid, one of the compounds identified in DSE inhibited *E. coli* biofilms by regulating *pga*ABCD gene expression. It reduced the synthesis of proteins, polysaccharides, and DNA in the biofilm matrix (Kang *et al.,* 2018). Similarly, a study reported that gallic acid affects *S. aureus* biofilm formation by down-regulating the *icaA* and *icaD*, genes that encode an enzyme involved in synthesizing the poly-N-acetylglucosamine polymer (Liu *et al.,* 2017).

These findings suggest that date seed extracts have significant potential as antibiofilm agents, which could be beneficial in treating infections and reducing contamination related to microbial biofilms. Although studies have been carried out to explore the antimicrobial

potential of the Mejhoul date seed, it is important to highlight that research on inhibiting bacterial biofilms using this cultivar is practically non-existent. Addressing this knowledge gap could reveal new strategies to combat bacterial biofilm formation in agriculture, healthcare, and the food industry, where bacterial resistance is a growing challenge, and the search for effective solutions is essential. Besides, these results underscore the significant impact of the San Luis Rio Colorado Valley's unique environmental conditions, which enhance the Mejhoul date seeds' bioactive potential. This highlights the importance of region-specific studies in understanding the full potential of agricultural byproducts for antimicrobial applications.

5. CONCLUSION

This study demonstrates the potential of mexican Mejhoul date seed as a natural antibacterial and antibiofilm agent against pathogenic bacteria. The extract, rich in phenolic and flavonoid compounds such as gallic and cinnamic acids, exhibited effective inhibitory activity against the important pathogens *E. coli*, *P. aeruginosa*, and *S. aureus*. These findings suggest that date seed extract could serve as an alternative to traditional antibiotics and synthetic disinfectants, helping to mitigate antibiotic resistance.

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AUTHOR CONTRIBUTION

Gutiérrez-Pacheco, M. M.: Conceptualization, Methodology, Data Curation, Funding, Research, Redaction-Original Draft. Salomón-Torres, R.: Conceptualization, Resources, Redaction-Review and Editing. Gutiérrez-Pacheco, S. L.: Methodology, Data Curation, Resources, Redaction-Review and Editing. Bernal-Mercado, A. T.: Resources, Methodology, Redaction-Review and Editing. Ayala-Zavala, J. F.: Resources, Visualization, Redaction-Review and Editing. González-Aguilar, G. A.: Resources, Methodology. Ortega-Ramirez, L. A.: Conceptualization, Data Curation, Redaction-Review and Editing, Funding, Research, Project Management.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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