

Comparative In-Vitro Efficacy of *Chenopodium quinoa* Seed and *Eriobotrya japonica* Fruit Extracts Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

The rise of antimicrobial resistance, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA), necessitates the exploration of novel therapeutic agents from accessible and affordable sources. This study provides a direct comparative evaluation of the in-vitro antimicrobial efficacy of methanolic extracts from commercially available *Chenopodium quinoa* seeds and *Eriobotrya japonica* fruit against a clinical MRSA isolate. Plant materials were purchased from a local supermarket. Extracts were prepared using Soxhlet extraction with 100% methanol. The antimicrobial activity was assessed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using the broth microdilution method according to CLSI guidelines. Data were analyzed using independent samples t-tests. The *E. japonica* fruit extract demonstrated significantly stronger anti-MRSA activity than the *C. quinoa* seed extract. The mean MIC and MBC for *E. japonica* fruit were $126.0 \pm 2.8 \mu\text{g/mL}$ and $251.0 \pm 2.4 \mu\text{g/mL}$, respectively. In contrast, *C. quinoa* seeds exhibited a mean MIC of $501.6 \pm 5.3 \mu\text{g/mL}$ and an MBC of $1005.0 \pm 10.0 \mu\text{g/mL}$. The differences were statistically significant ($p < 0.0001$), with the *E. japonica* fruit extract being approximately 4-fold more potent. The antimicrobial effects were dose-dependent. The methanolic fruit extract of *Eriobotrya japonica*, a commercially available product, is a potent source of bactericidal compounds against MRSA and shows significantly greater promise than *Chenopodium quinoa* seeds for the development of new phytotherapeutic agents to combat antibiotic-resistant infections. The accessibility and affordability of loquat fruit make it particularly attractive for large-scale development.

Introduction

The escalating crisis of antimicrobial resistance (AMR) poses a formidable threat to global public health, undermining decades of medical progress and threatening to return society to a pre-antibiotic era [1]. The World Health Organization has identified AMR as one of the top 10 global public health threats facing humanity, with projections indicating that drug-resistant diseases could cause 10 million deaths each year by 2050 if no action is taken [2]. Among the most notorious multidrug-resistant pathogens is Methicillin-resistant *Staphylococcus aureus* (MRSA), a leading cause of severe hospital- and community-acquired infections, including bacteremia, pneumonia, and skin and soft tissue infections [3]. The limited efficacy of conventional antibiotics against MRSA has created an urgent need for the discovery and development of novel therapeutic agents from alternative sources. Natural products, particularly those derived from medicinal plants and food crops, have historically been a rich reservoir of bioactive compounds and have served as the foundation for many modern pharmaceuticals [4].

Plants and plant-derived foods have evolved sophisticated chemical defense systems, producing a vast array of secondary metabolites to protect themselves from pathogens and environmental stress. This chemical diversity offers a promising avenue for identifying novel antimicrobial agents with unique mechanisms of action that can circumvent existing resistance pathways [5]. The exploration of ethnobotanical knowledge, the scientific validation of traditional plant-based remedies, and the investigation of commonly consumed food crops are therefore critical strategies in the search for new anti-MRSA therapies [6]. *Chenopodium quinoa* Willd. (quinoa) is a pseudocereal native to the Andean region that has gained global recognition for its exceptional nutritional value and has become a staple in health-conscious diets worldwide. Beyond its nutritional significance, quinoa seeds have been used in traditional medicine for centuries to treat a variety of ailments [7]. Scientific investigations have revealed that quinoa seeds are a rich source of bioactive compounds, including saponins, phenolic acids, flavonoids, and amino acids, which exhibit a broad spectrum of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties [8] [9]. Several studies have demonstrated the potential of quinoa seed extracts to inhibit the growth of various pathogenic bacteria, suggesting their utility as a source of antimicrobial agents [10].

Eriobotrya japonica (Thunb.) Lindl., commonly known as loquat or medlar, is an evergreen tree native to

East Asia that is widely cultivated for its fruit. The fruit has a long history of use in traditional Chinese medicine and folk remedies for the treatment of respiratory ailments such as cough and asthma [11]. Phytochemical studies have shown that *E. japonica* fruit is abundant in bioactive compounds, particularly organic acids, polyphenols, and carotenoids, which are known to possess potent anti-inflammatory, antioxidant, and antiviral properties [12, 13]. More recently, research has begun to uncover the significant antibacterial potential of *E. japonica* fruit extracts, with some studies reporting activity against various pathogenic bacteria, including MRSA [14].

The accessibility and widespread availability of both quinoa seeds and loquat fruit in commercial markets make them particularly attractive candidates for the development of affordable and sustainable antimicrobial agents. Unlike rare medicinal plants that require specialized cultivation, these food crops are readily available in local stores and markets worldwide, facilitating large-scale production and application. Despite the individual promise shown by both *C. quinoa* seeds and *E. japonica* fruit, there is a lack of direct comparative studies evaluating their efficacy against clinically relevant pathogens like MRSA. A systematic comparison is essential to identify the more promising candidate for further development. Therefore, this study was designed to provide a head-to-head evaluation of the *in vitro* antimicrobial activity of methanolic extracts from *C. quinoa* seeds and *E. japonica* fruit against a clinical isolate of MRSA. By determining and comparing their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, this research aims to elucidate which of these two commercially available food products holds greater potential as a source for novel anti-MRSA agents.

Methods

Plant Material and Extraction

Chenopodium quinoa seeds and *Eriobotrya japonica* fruit were purchased from a local supermarket in [Sebha/Libya] in May 2023. The quinoa seeds were of commercial food-grade quality, and the loquat fruit was obtained from the fresh produce section. The authenticity of both materials was confirmed by visual inspection and comparison with botanical reference materials in the Botany Department, Faculty of Science. The quinoa seeds were rinsed with distilled water and air-dried at room temperature ($25 \pm 2^\circ\text{C}$) for 5 days. The loquat fruit was washed with distilled water, the seeds were removed, and the flesh was cut into small pieces and air-dried at room temperature for 10 days until completely desiccated. The dried quinoa seeds (100 g) and loquat fruit flesh (100 g) were separately ground into fine powder using a laboratory mill. Each powdered material was subjected to extraction using 100% methanol (HPLC grade, Sigma-Aldrich, St. Louis, MO, USA) in a Soxhlet apparatus (Pyrex, USA) at a 1:10 (w/v) ratio. The extraction was performed continuously for 6 hours at room temperature. The resulting extracts were filtered through Whatman No. 1 filter paper, followed by a $0.45 \mu\text{m}$ membrane filter. The solvent was evaporated under reduced pressure using a rotary evaporator (Buchi, Switzerland). The final yields of the crude extracts were 12.5% (w/w) for *C. quinoa* seeds and 8.8% (w/w) for *E. japonica* fruit. The dried extracts were stored in amber glass bottles at -20°C until further use.

Phytochemical Composition Analysis

The qualitative phytochemical composition of the plant extracts was determined using standard phytochemical screening tests and high-performance liquid chromatography (HPLC) analysis [8,12].

Phytochemical Screening Tests

Qualitative phytochemical screening was performed on the methanolic extracts of both *Chenopodium quinoa* seeds and *Eriobotrya japonica* fruit using standard protocols to identify the presence of major phytochemical classes.

Phenolic Acids and Flavonoids

The presence of phenolic compounds was confirmed using the Folin-Ciocalteu reagent. Briefly, 0.5 mL of extract (1 mg/mL in methanol) was mixed with 2.5 mL of Folin-Ciocalteu reagent and 2 mL of sodium carbonate solution (7.5% w/v). The mixture was incubated at room temperature for 30 minutes, and the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). Gallic acid (Sigma-Aldrich) was used as a standard for quantification.

Saponins

The presence of saponins was detected using the foam test. Extract (2 mL, 1 mg/mL in distilled water) was vigorously shaken in a test tube for 15 seconds. The formation of stable foam persisting for at least 15 minutes indicated the presence of saponins.

Triterpenes

Triterpenes were identified using the Liebermann-Burchard test. Extract (1 mL, 10 mg/mL in chloroform) was treated with 1 mL of acetic anhydride and 2 drops of concentrated sulfuric acid. The development of a brown-red color indicated the presence of triterpenes.

Tannins

The presence of tannins was confirmed using the ferric chloride test. Extract (1 mL, 1 mg/mL in distilled water) was treated with 2-3 drops of 5% ferric chloride solution. The development of a blue-black or dark green color indicated the presence of tannins.

Carotenoids

Carotenoids were detected by dissolving the extract (10 mg) in 10 mL of petroleum ether. The development of a yellow-orange color indicated the presence of carotenoids. Tocopherols: The presence of tocopherols was confirmed using the Emmerie-Engel test. Extract (1 mL, 10 mg/mL in ethanol) was treated with 1 mL of ferric chloride solution (0.1 M) and 1 mL of α,α' -dipyridyl solution (0.1 M in ethanol). The development of a red color indicated the presence of tocopherols.

High-Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis was performed to identify and quantify specific phytochemical compounds in the extracts. The analysis was conducted using a Shimadzu HPLC system (Shimadzu, Japan) equipped with a UV-Vis detector (SPD-20A, Shimadzu) and a C18 reverse-phase column (250 mm \times 4.6 mm, 5 μ m particle size; Phenomenex, USA). Mobile Phase and Gradient: The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient elution was as follows: 0-5 minutes, 10% B; 5-30 minutes, 10-50% B; 30-40 minutes, 50-100% B; 40-45 minutes, 100% B; and 45-50 minutes, 100-10% B. The flow rate was maintained at 1 mL/min, and the injection volume was 20 μ L. Detection and Identification: Compounds were detected at 280 nm for phenolic acids and flavonoids, and at 254 nm for other polyphenolic compounds. Individual compounds were identified by comparison with retention times and UV absorption spectra of standard compounds (Sigma-Aldrich, USA), including gallic acid, chlorogenic acid, rosmarinic acid, quercetin, kaempferol, and naringenin. For triterpenes, a separate HPLC method was employed using a gradient of methanol and water (85:15 to 100:0) with detection at 210 nm. Quantification: Peak areas were used for quantification using external standard calibration curves. Results were expressed as mg of compound per gram of dried extract (mg/g).

Relative Abundance Assessment

The relative abundance of each phytochemical class was assessed based on the combined results of the phytochemical screening tests and HPLC quantification. A semi-quantitative scale was used to classify the abundance levels as follows: Absent (-), Trace (\pm), Low (+), Moderate (++) and High (+++). This classification was based on the intensity of color development in screening tests and the concentration values obtained from HPLC analysis.

Bacterial Strain and Culture Conditions

A clinical isolate of Methicillin-resistant Staphylococcus aureus (MRSA) was obtained from the culture collection of the Department of Medical Laboratory, Faculty of Medical Technology, [Sebha University]. The strain's identity was confirmed using standard biochemical tests, and its resistance profile was verified by antibiotic susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The quality control strain S. aureus ATCC 25923 was used for validation in all experiments. Bacterial cultures were maintained on Mueller-Hinton Agar (MHA; Oxoid Ltd., Basingstoke, UK) and sub-cultured every two weeks. For experiments, an inoculum was prepared by growing the bacteria in Mueller-Hinton Broth (MHB; Oxoid Ltd.) overnight at 37°C. The bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 colony-forming units (CFU)/mL, and then diluted to achieve a final inoculum size of 5×10^5 CFU/mL in the test wells.

Antimicrobial Susceptibility Testing

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the plant extracts against MRSA were determined using the broth microdilution method, following the CLSI M07 standard [15]. The experiments were conducted in sterile 96-well polystyrene microtiter plates (Corning Inc., Corning, NY, USA). Stock solutions of the extracts were prepared in 10% dimethyl sulfoxide (DMSO). Two-fold serial dilutions of each extract were prepared in MHB to achieve final concentrations ranging from 31.25 to 1000 μ g/mL. Each well contained 100 μ L of the diluted extract and 100 μ L of the standardized bacterial inoculum. Vancomycin (Sigma-Aldrich) was used as a positive control (1-16 μ g/mL). Wells containing MHB with the bacterial inoculum but without any extract served as a negative control, and wells with MHB and

10% DMSO were included to ensure the solvent had no inhibitory effect. The plates were incubated aerobically at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract that resulted in complete inhibition of visible bacterial growth. To determine the MBC, 10 µL aliquots were taken from all wells showing no visible growth and sub-cultured onto MHA plates. The plates were incubated at 37° C for 24 hours. The MBC was defined as the lowest concentration of the extract that resulted in a ≥99.9% reduction in the initial CFU count. All experiments were performed in five independent replicates.

Statistical Analysis

All data were expressed as the mean ± standard deviation (SD) from five independent experiments. The statistical significance of the differences between the MIC and MBC values of the two extracts was determined using an independent samples t-test. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (Version 26.0, IBM Corp., Armonk, NY, USA).

Results

Antimicrobial Activity of Plant Extracts

The antimicrobial activities of the methanolic extracts of *Chenopodium quinoa* seeds and *Eriobotrya japonica* fruit against the clinical isolate of MRSA were evaluated by determining their Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. The results are summarized in Table 1. The *E. japonica* fruit extract exhibited significantly stronger antimicrobial activity compared to the *C. quinoa* seed extract. The mean MIC for *E. japonica* fruit was 126.0 ± 2.8 µg/mL, while the mean MIC for *C. quinoa* seeds was 501.6 ± 5.3 µg/mL. Similarly, the mean MBC for *E. japonica* fruit was 251.0 ± 2.4 µg/mL, which was substantially lower than the 1005.0 ± 10.0 µg/mL observed for *C. quinoa* seeds.

Table 1. Antimicrobial Susceptibility Results Against MRSA

Extract Source	Parameter	Mean ± SD (µg/mL)	Range (µg/mL)
<i>Chenopodium quinoa</i> (Seeds)	MIC	501.6 ± 5.3	495-510
<i>Chenopodium quinoa</i> (Seeds)	MBC	1005.0 ± 10.0	990-1020
<i>Eriobotrya japonica</i> (Fruit)	MIC	126.0 ± 2.8	122-130
<i>Eriobotrya japonica</i> (Fruit)	MBC	251.0 ± 2.4	248-255

Note: MIC, Minimum Inhibitory Concentration; MBC, Minimum Bactericidal Concentration. Values represent mean ± standard deviation of five replicates. MRSA, Methicillin-resistant *Staphylococcus aureus*.

Dose-Response Relationship

The inhibitory effects of both extracts were found to be concentration-dependent, as illustrated by the dose-response curves in (Figure 1). The percentage of bacterial growth inhibition increased with rising concentrations of both extracts. The *E. japonica* fruit extract demonstrated a steeper dose-response curve, achieving over 90% inhibition at a concentration of 250 µg/mL. In contrast, the *C. quinoa* seed extract required a concentration of 1000 µg/mL to achieve a comparable level of inhibition.

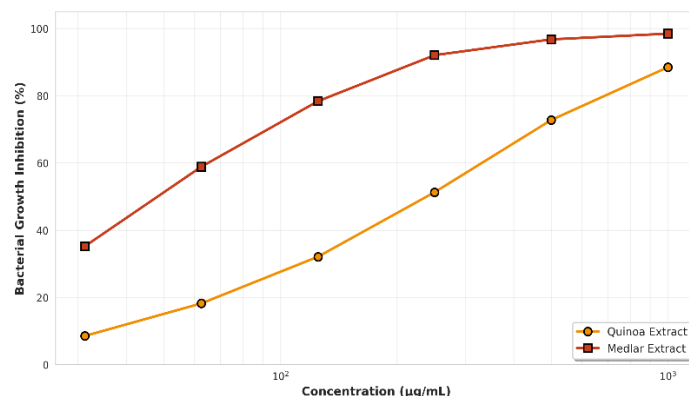


Figure 1. Dose-response curves showing the percentage of MRSA growth inhibition at various concentrations of *Chenopodium quinoa* seed and *Eriobotrya japonica* fruit extracts

Statistical Analysis

The differences in the antimicrobial activities between the two extracts were statistically significant. An independent samples t-test revealed a highly significant difference in both MIC (t = 125.48, p < 0.0001) and MBC (t = 146.75, p < 0.0001) values, as detailed in (Table 2). The *E. japonica* fruit extract was approximately 4-fold more potent than the *C. quinoa* seed extract in both inhibiting and killing MRSA.

Phytochemical Composition

A qualitative analysis of the phytochemical composition of the extracts revealed distinct profiles for each plant material, as shown in Table 2. The *E. japonica* fruit extract was rich in triterpenes, organic acids, and tannins, while the *C. quinoa* seed extract was characterized by the presence of saponins. Both extracts contained notable amounts of phenolic acids and flavonoids.

Table 2. Phytochemical Composition of Plant Extracts

Phytochemical Class	<i>Chenopodium quinoa</i> Seeds	<i>Eriobotrya japonica</i> Fruit
Phenolic Acids	Rosmarinic acid, Chlorogenic acid, Gallic acid	Gallic acid, Chlorogenic acid, Citric acid
Flavonoids	Quercetin, Isoquercetin, Kaempferol	Quercetin, Kaempferol 3-O-β-glucoside, Naringenin
Organic Acids	Trace amounts	Citric acid, Malic acid, Tartaric acid
Triterpenes	Trace amounts	Oleanolic acid, Ursolic acid, Corosolic acid
Saponins	Present	Absent/Trace
Tannins	Low levels	Moderate levels
Carotenoids	Trace amounts	Moderate levels
Tocopherols	α-tocopherol, β-tocopherol	Trace amounts

Discussion

The emergence of multidrug-resistant pathogens, particularly Methicillin-resistant *Staphylococcus aureus* (MRSA), represents a significant threat to global public health, necessitating the urgent discovery of novel antimicrobial agents [2]. This study evaluated the *in vitro* antimicrobial potential of methanolic extracts from *Chenopodium quinoa* seeds and *Eriobotrya japonica* fruit against a clinical MRSA isolate. The principal finding of this research is that the *E. japonica* fruit extract demonstrated substantially greater antimicrobial efficacy than the *C. quinoa* seed extract. This result is both statistically significant and clinically relevant. The *E. japonica* fruit extract exhibited a potent bactericidal effect, with a mean MIC of 126.0 µg/mL and a mean MBC of 251.0 µg/mL. These values are noteworthy and compare favorably with those reported for other plant-derived antimicrobials. The observed potency is likely attributable to the rich phytochemical profile of *E. japonica* fruit, which is known to contain high concentrations of organic acids (citric, malic, and tartaric acids), polyphenolic compounds, and triterpenes (oleanolic acid, ursolic acid) [12,13]. These classes of compounds are well-documented for their antimicrobial properties, often mediated by mechanisms such as cell membrane disruption, enzyme inhibition, and substrate deprivation [5]. The organic acids present in loquat fruit are particularly notable for their ability to lower pH and create an inhospitable environment for bacterial growth, while the triterpenes contribute to direct antimicrobial activity through multiple mechanisms [12].

The approximately 4-fold higher potency of the *E. japonica* fruit extract compared to the *C. quinoa* seed extract underscores its potential as a source for novel anti-MRSA compounds and highlights the value of commonly available fruit in antimicrobial drug discovery. In contrast, the *C. quinoa* seed extract showed moderate activity, with MIC and MBC values of 501.6 µg/mL and 1005.0 µg/mL, respectively. While less potent than *E. japonica* fruit, these results are consistent with previous reports that have established the antimicrobial properties of quinoa seed extracts against various pathogens [9,10]. The activity of the *C. quinoa* seed extract can be linked to its distinct phytochemical composition, which includes saponins, phenolic acids (particularly rosmarinic and chlorogenic acids), and flavonoids [8]. Saponins, in particular, are known to possess antimicrobial activity by interacting with bacterial cell membranes and causing pore formation, leading to leakage of cellular contents [16]. The phenolic acids and flavonoids in quinoa seeds also contribute to antimicrobial activity through their ability to generate reactive oxygen species and inhibit essential bacterial enzymes [8]. The difference in efficacy between the two extracts likely reflects the different concentrations and types of bioactive compounds present in seeds versus fruit, as suggested by our qualitative phytochemical analysis (Table 3). The use of commercially available food products—quinoa seeds and loquat fruit purchased from local stores—represents a significant advantage for practical application and scalability. Unlike rare medicinal plants that require specialized cultivation and may face sustainability concerns, these food crops are widely available, affordable, and already integrated into global food supply chains. This accessibility makes them particularly attractive for the development of cost-effective antimicrobial agents that could be implemented in resource-limited settings. The fact that commercially available products demonstrate such strong antimicrobial activity suggests the potential for direct application in functional food development or as starting materials for pharmaceutical development.

This study has several strengths, including the use of standardized CLSI methodology for antimicrobial

susceptibility testing, the inclusion of a quality control reference strain, and the robust statistical analysis of data from multiple replicates. The use of commercially available materials also enhances the reproducibility and practical applicability of the findings. However, some limitations must be acknowledged. The findings are based on in vitro experiments, and the efficacy of these extracts in vivo remains to be determined. Furthermore, this study utilized crude extracts, and the specific compounds responsible for the observed anti-MRSA activity have not been isolated and characterized. The investigation was also limited to a single clinical isolate of MRSA, and the activity against a broader panel of strains may vary. Additionally, the commercial sourcing of materials means that variations in harvest time, storage conditions, and processing may affect the phytochemical composition and antimicrobial activity of future batches. Based on these findings, several avenues for future research are warranted.

The most promising next step is the bioassay-guided fractionation of the *E. japonica* fruit extract to isolate and identify the specific active compounds responsible for its potent anti-MRSA activity. Subsequent studies should evaluate the efficacy of these purified compounds against a diverse panel of clinical MRSA isolates, including both hospital-acquired and community-acquired strains. Investigating the precise mechanism of action through techniques such as scanning and transmission electron microscopy would provide valuable insights into how these compounds kill MRSA. Finally, preclinical studies, including cytotoxicity assays on human cell lines and in vivo efficacy and safety evaluations in animal models, are essential to assess the therapeutic potential of these natural products. Studies examining the stability and bioavailability of the active compounds under physiological conditions would also be important for translating these findings into clinical applications.

Conclusion

This study provides compelling evidence that the methanolic fruit extract of *Eriobotrya japonica*, a commercially available product, is a potent source of bactericidal compounds against MRSA. Its superior activity compared to *Chenopodium quinoa* seeds highlights its potential for the development of new phytotherapeutic agents or as a source for lead compounds in the fight against antibiotic-resistant bacteria. The accessibility and affordability of loquat fruit make it a particularly promising candidate for further development as a natural antimicrobial agent for both pharmaceutical and functional food applications.

Conflict of interest. Nil

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