Research article

Antioxidant and Antibacterial Activities of *Jasminum officinale* L. f. var. *grandiflorum* (L.) Kob. Leaf Extracts

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Abstract

The investigation of phytochemicals and their effects on plants is currently receiving significant attention due to the well-known fact that plants contain secondary metabolites that have high levels of usefulness in various applications. These applications include food additives, biopesticides, and pharmaceuticals. In a recent study, we thoroughly examined the total phenolic content (TPC), and the antioxidant, and antibacterial properties of *Jasminum officinale* L.f. var. *grandiflorum* (L.) Kob. (also known as Spanish jasmine) leaf extracts, providing valuable insights into its potential benefits. Our study performed a crude methanol (ME) extraction of Spanish jasmine leaves with acid-base solvent partitioning, resulting in an acidic fraction (AE), a neutral fraction (NE), and an aqueous fraction (AQ). The Folin-Ciocalteu method was used to determine the TPC of the extracts. The AE fraction displayed the highest TPC (79.8±0.61 mgGAE/g dry weight), followed by the NE and AQ fractions. The extracts were further evaluated for their antioxidant activity using the DPPH assay. The AE fraction had the highest antioxidant activity with an EC_{50} value of 4.6±0.40 µg/mL. The main active compound, oleuropein, was isolated and determined using spectral data. The isolated compound displayed excellent antioxidant activity with an EC_{50} value of 13.0±0.06 µg/mL. Moreover, the antibacterial activity of the Spanish jasmine leaf extracts was calculated using the disc diffusion method based on the minimum inhibitory concentration. The AE fraction exhibited activity against the Gram-negative bacteria, *Vibrio parahaemolyticus*, with a MIC value of 0.78 µg/mL. The AE fraction also displayed the highest degree of activity against *Staphylococcus* strains, including drug-resistant

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strains (MRSA and VRSA), with a MIC range of 0.39-12.5 µg/mL. It is important to highlight that the AE fraction demonstrated the highest level of activity against all VRSA strains tested despite their resistance to vancomycin and oxacillin. These findings strongly indicate that Spanish jasmine leaves possess potent natural antioxidant and antibacterial properties, positioning them as a highly promising plant source.

1. Introduction

It is important to acknowledge that many people worldwide, especially in developing countries, rely on natural plant-based remedies as their main source of healthcare. These medicinal plants are essential in providing crucial healthcare services, and they are used to make various products, such as pharmaceuticals, fragrances, and biopesticides. The phytochemicals produced through the secondary metabolism of these plants exhibit an impressive level of chemical and structural complexity and are widely acknowledged for their beneficial properties [1, 2]. Lately, research has been dedicated to discovering antioxidant and antibacterial elements in plants that can be utilized in the production of food, cosmetics, and pharmaceuticals. Experts are especially interested in finding natural antioxidants in plant materials because they are considered safer than synthetic antioxidants. Some synthetic antioxidants have been found to negatively impact human health, so natural alternatives are being explored. In addition, biological antimicrobial agents are crucial for safeguarding living organisms against different diseases [3, 4].

Jasminum spp. plants are a highly esteemed and widely adored group of flowering plants, that are prized for their alluring fragrance and breathtaking blossoms. As members of the Oleaceae family, their leaves, stems, bark, and roots are highly regarded in the pharmaceutical industry. For instance, *Jasminum sambac* is a versatile plant that effectively addresses various health concerns such as skin conditions, ulcers, and fever [1]. Its essential oil is also widely used in skin care products as a fragrance component [4]. One *Jasminum* species commonly used in folk medicine is *J. officinale* L.f. var. *grandiflorum* (L.) Kob., also known as Spanish jasmine. This plant is a well-known glabrous twining shrub widely cultivated in China, India, the Mediterranean, Northern Persia, and Pakistan [5-7]. Its flowers and leaves are known for their therapeutic proprties such as antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant, and tonic effects. The flowers can also be used for wound care, infections, and mental diseases. The essential oil extracted from the flowers of Spanish jasmine reduces skin inflammation, helps skin tone, and lifts mood [8, 9]. In South China, Spanish jasmine buds have long been utilized as a natural remedy to alleviate health issues such as dysmenorrhea, hepatitis, stomatitis, and duodenitis. This traditional treatment has been used for a considerable time [10]. In addition, it has been revealed that Spanish jasmine leaves possess exceptional allelopathic properties. The methanolic extract derived from these leaves can remarkably impede the growth of weeds such as *Echinochloa crus-galli* (L.) Beauv. and *Phaseolus lathyroides* L. by stunting their root and shoot length and hindering seed germination. This significant finding highlights the potential of Spanish jasmine leaves as a natural weed control solution [11, 12].

Previous research has demonstrated the impact of Spanish jasmine leaf methanolic extract on monocot and dicot plants *via* allelopathy [11, 12]. Our most recent investigation delves further into the extract's secondary activities through the evaluation of distinct fractions, acidic (AE), neutral (NE), and aqueous (AQ) for their antioxidant and antibacterial properties. We discovered that the fractions derived from the methanolic extract displayed remarkable efficacy, emphasizing the significance of analyzing individual fractions for their possible advantages.

2. Materials and Methods

2.1 General experimental procedures

An FT-NMR analysis was conducted to confirm the structure and purity of the identified compounds. A Bruker AVANCE 300 NMR spectrometer, operating at 300 MHz and 75.5 MHz, provided the ^{1}H and ^{13}C NMR spectra. Merck silica gel 60 (<0.063 mm) and Scharlau GE 0048 silica gel 60 (0.02-0.06 mm) were used for flash column chromatography (FCC) and quick column chromatography (QCC), respectively. Merck precoated silica gel 60 F_{254} plates were utilized for thin layer chromatography (TLC), and the spots were visualized under UV light. An anisaldehyde-H_2SO_4 reagent was sprayed on the spots, followed by heating.

2.2 Plant material

Spanish jasmine leaves were gathered from a production field in Supan Buri province, Thailand. The identification process was conducted by Dr. Chamroon Laosinwattana, an Associate Professor from the Department of Plant Production Technology at the School of Agricultural Technology in KMITL, Bangkok, Thailand. The leaves were then harvested, thoroughly cleaned with tap water, air-dried, and oven-dried for five days at 45°C.

2.3 Preparation of plant extracts

To obtain the ME crude extract, a kilogram of Spanish jasmine powdered leaves underwent a 5-day extraction process with 90% methanol, using three 5-liter batches at room temperature. The resulting mixture was then filtered through three layers of cheesecloth to eliminate any debris before being filtered again through Whatman No. 1 filter paper. Finally, the extract was evaporated under reduced pressure to yield a sticky residue. Afterward, the remaining substance was mixed with 500 mL of distilled water and stirred vigorously using a magnetic stirrer for 30 min. This process resulted in an aqueous solution that was then acidified to pH 3 using 6 N HCl. The solution was filtered and extracted with ethyl acetate three times (3 x 500 mL). Subsequently, the aqueous phase was dried under reduced pressure at 45°C, resulting in an AQ fraction. The ethyl acetate solutions were mixed and then processed with anhydrous sodium sulfate. They were then concentrated to about 500 mL and subjected to three extractions with saturated aqueous sodium bicarbonate (3 x 500 mL). The ethyl acetate phase was dried using anhydrous sodium sulfate and concentrated under reduced pressure, resulting in the NE fraction, which was a neutral fraction soluble in ethyl acetate. The saturated aqueous sodium bicarbonate was evaporated until it reached approximately 1 L in volume. Then, the pH was adjusted to a level of 7 using 6 N HCl. Next, the mixture was extracted with ethyl acetate (3 times with 500 mL each). The resulting ethyl acetate solutions were then combined, and the moisture was removed over anhydrous sodium sulfate. Finally, the solution was evaporated under reduced pressure to obtain the ethyl acetate-soluble acidic fraction, also known as the AE fraction. The residue of the liquid mixture was discarded [11]. The three fractions obtained (AE, NE, and AQ) were examined for their abilities to act as antioxidants and antibacterial agents. The fraction that displayed the strongest activity was separated and subjected to column chromatography, which led to the isolation of an active compound. The active compound was then analyzed in detail using spectroscopic methods.

2.4 Isolation and identification of compounds

Twenty grams of the AE fraction was fractionated in a Silica gel 60 column using an *n*-hexane/ethyl acetate gradient and methanol. Each fraction was collected and evaporated under a vacuum, then pooled based on the TLC analysis in nine fractions labeled AE1 to AE 9 [eluted with *n*-hexane (AE1), *n*-hexane-EtOAc 80:20 (AE2), *n*-hexane-EtOAc 70:30 (AE3), *n*-hexane-EtOAc 65:35 (AE4), *n*-hexane-EtOAc 60:40 (AE5), EtOAc 50:50 (AE6), *n*-hexane-EtOAc 30:70 (AE7), *n*-hexane-EtOAc 20:80 (AE8), EtOAc-MeOH 95:5 (AE9) and MeOH].

Fifteen grams of fraction AE9 was subjected to column chromatography using silica gel 60. AE9 was eluted with an EtOAc-MeOH (98:2) gradient to obtain a compound identified as oleuropein, which is a secoiridoid glucoside, (Figure 1).

2.5 Bioassays

2.5.1 Determination of total phenolic compounds

The phenolic content in the sample was measured in milligrams using the Folin-Ciocalteu reagent method [13]. Three replicates of 50 µL of the sample were added to 2 mL of a 1/10 dilution of Folin-Ciocalteu reagent and 2 mL of Na_2CO_3 (7.5%, w/v). These were incubated at 37°C for 15 min. The absorbance of the samples was then measured at 765 nm using a Thermo Spectronic-Genesys 10 series spectrophotometer. The results were expressed as gallic acid equivalent per gram of dry weight (mg GAE/g dw).

2.5.2 Determination of antioxidant activity

The antioxidant activity of the fractions was measured in terms of the hydrogen-donating or radical scavenging ability using the stable radical 1,1-diphenyl-2-picryhydrazyl (DPPH, Sigma-Aldrich, Germany) assay. Each sample extract (10 mg/mL) was mixed with 2.9 mL of DPPH radical solution in ethanol (4.5% v/v). The reaction mixtures were shaken vigorously and incubated in the dark for 30 min. The absorbance of the solution was measured at 517 nm.

Percentage DPPH inhibition was calculated using the formula;

$$\text{DPPH inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The scavenging effect was determined by comparing the absorbance of the solution containing the test sample to that of the control solution without the test sample. The percentage of the remaining DPPH against the standard concentration was plotted to obtain the amount of antioxidants necessary to decrease the effective DPPH concentration by 50% (EC_{50}). The standard antioxidant 2,6-di-(*tert*-butyl)-4-methyl phenol (BHT, EC_{50} value of 6.2±0.29 µg/mL) was used as a positive control.

2.5.3 Bacterial strains

This study utilized a total of twenty-three microorganisms, including *Vibrio cholerae*, *V. parahaemolyticus* (Gram-negative bacteria), *Bacillus subtilis* ATCC 26633, *Bordetella pertussis*, *Corynebacterium diphtheriae*, *Streptococcus milleri* group, *S. mutans* ATCC 27175, *S. pneumoniae*,

S. sobrinus, *Staphylococcus* coagulase-negative, *Staphylococcus aureus* ATCC 25923, *S. aureus* MRSA N1, and various strains of *S. aureus* MRSA and VRSA (Gram-positive bacteria). The Department of Stomatology, Faculty of Dentistry, Srinakharinwirot University, provided these standard strains.

2.5.4 Antibacterial activity

The disc diffusion method was utilized to test the antibacterial properties [14]. The efficiency of various fractions of Spanish jasmine leaves, and the isolated compounds from the said leaves were assessed against twenty-one strains of Gram-positive bacteria and two types of Gram-negative bacteria, as detailed in Table 2. The test was conducted by dissolving the fractions in dimethyl sulfoxide (DMSO) and making a two-fold serial dilution with the same solvent. Then, 0.1 mL of a bacterial suspension containing 10^5 to 10^6 CFU (colony forming unit/mL) was spread onto Mueller-Hinton agar plates. Sterile filter paper discs of 6 mm diameter were prepared and seeded with 10 µL of each test material in serial two-fold dilutions. These discs were placed on the plates and incubated at 37°C for 16-18 h. DMSO was used as a negative control to ensure accuracy, and vancomycin and oxacillin (E-test, AB Biodisk, Sweden) were used as positive controls. After the incubation period, the size of the inhibition zone was determined by measuring the distance from the disc's edge to the inner boundary of the surrounding pathogens. The diameter of the inhibitory zone was measured in three dimensions for each disc using a ruler with 1 mm divisions. Discs with a diameter of less than 8 mm were excluded for clarity [15]. The minimum inhibitory concentration (MIC) refers to the lowest concentration of the test samples that completely inhibits visible growth. The expressed unit for the MIC of the sample test is mg/mL.

2.6 Statistical analysis

Three replications were conducted to measure the total phenolic content and DPPH free radical scavenging activity of various fractions of the Spanish jasmine leaves. Data analysis was performed using IBM SPSS Statistics 25 software. Tukey's studentized range test was applied to compare means at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1 Plant extraction

The crude methanol (ME) extract of the Spanish jasmine leaves was subjected to acid-base solvent partitioning, successfully separating it into three distinct fractions: AE (6.79%), NE (12.60%), and AQ (8.12%). The NE fraction had the highest yield, followed by the AQ and AE fractions based on the percentage yields obtained from the crude methanol.

3.2 Total phenolic content

A Folin-Ciocalteu assay [13] was utilized to determine the total phenolic content of the fractions. This assay measures the electron transfer and resulting reducing capacity, which is then expressed as phenolic content. Table 1 displays the total phenolic content, measured in mg gallic acid equivalent per gram of dry weight, which varied from 25.0±0.60 mg GAE/g dw to 79.8±0.61 mg GAE/g dw. The results conclusively demonstrated that the AE fraction, extracted through acid-base solvent partitioning, exhibited the highest phenolic content at 79.8±0.61mg GAE/g dw. The NE

fraction showed a moderate activity level at 35.9±0.06 mg GAE/g dw. In comparison, the AQ fraction had the lowest total phenolic content. The NE fraction had a lower total phenolic content than the AE fraction. Noreen *et al*. [16] suggested that selecting solvent and extraction methods can affect both the total phenolic content and the yield of plant extracts. It has been scientifically observed that extraction with acid or base can significantly impact the phenolic content. Our research studies have unequivocally shown that preparing an AE fraction through base extraction is crucial to significantly enhancing phenolic compound solubility.

3.3 DPPH free radical scavenging activity

According to Apak *et al*. [17], it is not possible to determine antioxidant activity using just one test. In research studies, the antioxidant properties of plants are usually evaluated based on their ability to scavenge free radicals. Free radicals are known to contribute to various chronic diseases, such as cancer and cardiovascular disease. As a result, our study also included a DPPH assay, which relies on the plant's ability to donate hydrogen to scavenge DPPH radicals [18]. Various fractions of Spanish jasmine were assessed for their antioxidant properties and measured in terms of EC_{50}, which indicates the concentration to inhibit 50% of DPPH in the reaction mixture. BHT was used as a positive control. Table 1 shows that the AE fraction displayed the most potent DPPH radical inhibition ability, with an EC_{50} value of 4.6±0.40 µg/mL. The NE and AQ fractions followed with respective EC_{50} values of 11.0±0.05 µg/mL and 32.2±0.29 µg/mL. The AE fraction demonstrated an EC_{50} value that exhibited nearly the same value as that of BHT, which was 6.2±0.29 µg/mL. According to the study, the AE fraction had higher antioxidant activity than the NE fraction. This difference may be due to the presence of non-polar antioxidant compounds in the NE fraction [16]. Based on the findings of the oxidant properties analysis, it is evident that the acid-base extraction method generated extracts with different degrees of antioxidant capacity.

Table 1. Total phenolic content and antioxidant activity of various fractions of Spanish jasmine leaf

Fractions	Total phenolic content[a] (mg GAE/g dw)	Antioxidant activity[b] (EC_{50} µg/mL)
NE	35.9±0.06[a]	11.0±0.05[a]
AE	79.8±0.61[b]	4.6±0.40[b]
AQ	25.0±0.60[c]	32.2±0.29[c]

a The experiments were performed according to the Folin-Ciocalteu method;
b The experiment was performed using DPPH free radical scavenging assay.
Based on Tukey's test, different superscript letters in the same column are significantly different ($p < 0.05$).

Studies show that *Jasminum* plants possessed remarkable antioxidant properties. Organic solvents were used to extract compound from plant components such as stems, bark, leaves, roots, and flowers. The resulting extracts were analyzed for their antioxidant activity. In addition, the potential antioxidant effects of the essential oil derived from *Jasminum* flowers were also studied. In a study conducted by Shekhar and Prasad [19], the antioxidant activity of leaves of various *Jasminum* species was analyzed using the DPPH method and compared to a standard compound, ascorbic acid. The results indicated that the ethanolic extract exhibited a higher antioxidant activity than the methanolic extract. Specifically, the *J. auriculatum* sample extracted with ethanol showed the highest antioxidant activity, followed by *J. aungustifolium*. In contrast, the *J. sambac* cultivar

variety showed the lowest antioxidant activity, requiring 1300 µL of the sample to reduce DPPH to 50% from its initial concentration. The methanolic solvent extracted sample analysis revealed that *J. grandiflorum* exhibited the highest antioxidant activity, followed by the *J. sambac* wild variety. On the other hand, the *J. sambac* cultivar variety demonstrated the lowest antioxidant activity, as it needed 1100 µL of the sample to reduce DPPH to 50% of its initial concentration. Kumaresan *et al*. [20] explored the antioxidant properties of *J. multiflorum* leaves and flowers. The EC_{50} value was analyzed to determine the DPPH potential. The findings revealed that both the leaf and flower extracts of *J. multiflorum* exhibited inhibiting activity, with the highest inhibiting activity observed in the ethanolic extract of leaves (EC_{50} value of 141.2±1.24 µg/mL), while the flower extract had an EC_{50} value of 252.42±2.41 µg/mL. The antioxidant activity of *J. officinale* methanol and ethanol extracts was assessed using the hydrogen peroxide method. The results indicate that both extracts exhibited antioxidant activity, with the ethanolic extract showing a more potent effect [21]. In a study conducted by Galovičova *et al*. [22], the antioxidant activity of *J. grandiflorum* essential oil was examined, and the findings showed that the DPPH method indicated an inhibition rate of 58.47%, which was equivalent to 220.93 TEAC. Borar *et al*. [23] found that the methanolic leaf extract from *J. mesnyi* showed significant antioxidant potential in the DPPH assay through its ethyl acetate and *n*-butanol fractions, surpassing the IC_{50} values of ascorbic acid (IC_{50} 6.54 µg/mL) and rutoside (IC_{50} 5.44 µg/mL). Specifically, the *n*-butanol fraction displayed an impressive DPPH radical scavenging ability with an IC_{50} of 6.22 µg/mL, while the ethyl acetate fraction showed an IC_{50} value of 153.45 µg/mL. Mittal *et al*. [24] examined the antioxidant capabilities of the ethanol extract from *J. auriculatum* leaves. The results revealed that the ethanol extract from *J. auriculatum* exhibited significant DPPH scavenging activity, with an impressive IC_{50} value of 33.39 µg/mL and a total phenolic content of 8.47 mg GAE/g. Interestingly, the standard ascorbic acid had a lower IC_{50} value of 35.41 µg/mL in the DPPH scavenging assay compared to *J. auriculatum*. El-Hawary *et al*. [25] studied the antioxidant activity of different *Jasminum* species grown in Egypt. They evaluated the antioxidant activity of *J. azoricum* L., *J. humile* L., *J. multiflorum* (Burm.f.) Andrew, *J. officinale* L., *J. sambac* (Ait) L. "Arabian Nights cultivar," and *J. sambac* (Ait) L. "Grand Duke of Tuscany cultivar," using the DPPH assay. The results showed that *J. multiflorum* had the highest antioxidant activity among the species tested, with an IC_{50} of 34.8 µg/mL. *Jasminum officinale* also exhibited significant antioxidant activity compared to the other *Jasminum* species. According to a study by Guo *et al*. [26], the stem of *J. neroosum* contained Jasnervosides A-H, which was found to exhibit DPPH radical scavenging activity with inhibitory percentages ranging from 18.44 to 82.6%. Out of these compounds, Jasnervosides A, B, D, and G were particularly effective antioxidants, with IC_{50} values of 0.22, 0.09, 0.19, and 1.21 µg/mL, respectively.

In order to validate our findings, we utilized chromatography to purify the AE fraction, which amounted to 15 g. As a result, we isolated an active compound (534.9 mg), depicted in Figure 1, as a pale-yellow oil. The structure of oleuropein was elucidated by FTIR, ^{1}H-NMR, and ^{13}C-NMR analysis. Table 2 compares the compound's NMR data with previously reported data [27, 28] and identifies it as oleuropein, a secoiridoid glucoside. After thoroughly analyzing the isolated compound's antioxidant activity through the DPPH free radical scavenging system, the compound exhibited an impressive level of antioxidant activity, with an EC_{50} value of 13.0±0.06 µg/mL. These results demonstrated the compound's potential as a valuable antioxidant agent.

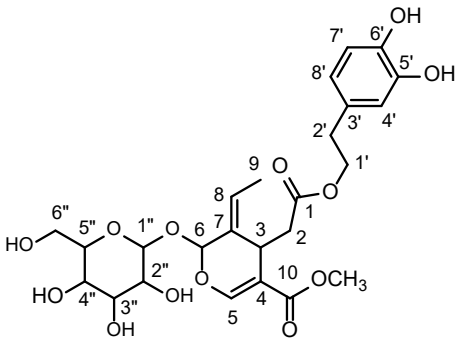

Figure 1. Chemical structure of oleuropein

Table 2. NMR spectroscopic data of oleuropein (δ in ppm, *J* in Hz)

Position	Oleuropein[a]		Oleuropein[b]	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	172.1	-	171.1
2	2.66, d	34.1	2.63, dd	34.2
3	3.85, dd	30.6	3.86, dd	30.6
4	-	108.1	-	108.2
5	7.47, s	154.1	7.52, s	153.9
6	5.87, s	94.1	5.87, s	93.6
7	-	129.6	-	129.7
8	6.04, q	123.8	5.96, q	123.5
9	1.69, d	12.5	1.65, d	13.4
10	-	167.6	-	166.6
OCH_3	3.67, s	51.1	3.66, s	51.7
1′	4.08, m	65.8	4.07, m	65.5
2′	2.72, t	45.1	2.69, t	45.6
3′	-	129.1	-	128.9
4′	6.70, d	115.9	6.61, d	116.6
5′	-	144.9	-	145.6
6′	-	143.6	-	144.2
7′	6.64, dd	115.3	6.63, dd	116.0
8′	6.51, dd	120.2	6.48, dd	120.0
1″	4.45, d	99.7	4.48, d	99.5
2″	3.06, m	73.5	3.08, m	73.8
3″	3.20, m	77.1	3.22, m	77.0
4″	3.08, m	70.2	3.10, m	70.5
5″	3.15, m	76.6	3.17, m	77.8
6″	3.68, m	61.5	3.68, m	61.6

a Recorded in CD_3OD, ^{1}H at 300 MHz, ^{13}C at 75 MHz

b Spectroscopic data was discovered within the literature [27, 28]. Recorded in DMSO-d_6, ^{1}H at 400 MHz, ^{13}C at 100 MHz

3.4 Antibacterial activity

Table 3 presents the MIC values of three fractions, AE, NE, and AQ, obtained from the methanolic extract of Spanish jasmine leaves. These fractions were evaluated against various pathogenic bacteria. The AE and NE fractions were effective, whereas the AQ fraction showed low activity against the experimental organisms. Our study has demonstrated the remarkable efficacy of the AE fraction in battling Gram-negative bacteria, including *V. cholerae* and *V. parahaemolyticus*. Notably, the MIC values for the AE fraction against these bacteria were 25 and 0.78 µg/mL, respectively. The AE fraction also displayed antibacterial activity against *S. sobrinus* and *S. aureus* ATCC 25923, both Gram-positive bacteria, with respective MIC values of 12.5 and 1.56 µg/mL. Furthermore, the AE fraction exhibited moderate to high antibacterial action against drug-resistant anti-MRSA and VRSA *S. aureus* strains, with MIC values ranging from 0.36 to 12.5 µg/mL. The efficacy of the AE fraction was potent, particularly in its ability to combat VRSA strains, surpassing even the antibacterial activity levels of vancomycin and oxacillin. The results obtained from the NE fraction indicated its effectiveness in combating the Gram-negative bacterial *V. parahaemolyticus*, with a MIC value of 1.56 µg/mL. However, it showed varying degrees of antibacterial activity against Gram-positive bacteria such as *S. aureus* ATCC 25923, *C. diphtheriae*, and *S. sobrinus*, with the MIC values ranging from 0.78 to 25 µg/mL. Additionally, the NE fraction exhibited antibacterial properties against the drug-resistant anti-MRSA and VRSA *S. aureus* strains, with MIC values ranging from 1.56 to 12.5 µg/mL. In contrast, the AE fraction proved to be more potent against the drug-resistant anti-MRSA and VRSA *S. aureus* strains compared to the NE fraction.

Jasminum spp. plants exhibit antimicrobial activity against both Gram-positive and Gram-negative bacterial strains, as well as fungal pathogens. These plants are highly effective in combating a wide range of bacterial pathogens. Thiruvengadam *et al*. [29] demonstrated that the acetone extract obtained from *J. azoricum* leaves displayed the highest activity against *S. aureus*. At a 30 mg/mL concentration, it produced an inhibition zone of 30 mm. In a study conducted by Al-Khazraji [4], the antibacterial properties of *J. officinale* were tested *in vitro* using the ethanolic extract of its different parts (flowers, stems with leaves, and roots). Four reference bacteria, namely *S. aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC25922, and *Pseudomonas aeruginosa* ATCC 27853, were used to evaluate the extract. The results revealed that the ethanolic extracts of the flowers and stems with leaves exhibited a MIC of 2 mg/mL against all the tested bacteria. On the other hand, the root extract displayed a MIC of 4 mg/mL against *S. aureus*, *E. faecalis*, and *E. coli*, while its MIC against *P. aeruginosa* was 2 mg/mL. Based on the findings of Ali *et al*. [30], the methanolic extract derived from the leaves of *J. grandiflorum* possessed antibacterial properties. The extract was subjected to the disc diffusion method at a concentration of 1000 µg/disc, and was tested against twelve Gram-positive and eighteen Gram-negative bacteria. The results revealed that the *J. grandiflorum* extract demonstrated potent activity against six Gram-positive (*B. anthracis*, *B. pumilus*, *C. diphtheriae*, *C. hoffmanii*, *C. xerosis*, and *S. citreus*) and one Gram-negative bacteria (*Branhamrlla catarrhalis*).

Our research shows that Spanish jasmine exhibits antibacterial properties, which is consistent with previous studies on the topic. Moreover, we conducted experiments on the isolated compound oleuropein to assess its antibacterial effects on experimental organisms. The results indicate that oleuropein displays moderate antibacterial activity against five Gram-positive bacteria, including *S. sobrinus*, four strains of *S. aureus* MRSA, and *S. aureus* VRSA, with MIC values ranging from 12.5 to 50 µg/mL. Studies demonstrate that polyphenol plants possess antimicrobial properties. This is achieved by disrupting the functions of bacterial cell membranes, which ultimately inhibits their growth and multiplication [31]. The AE fraction's antibacterial activity may be due to phenolic compounds and their synergistic effects with non-phenolic compounds found in the extracts [32].

Table 3. Antibacterial activities (MIC, µg/mL) of various Spanish jasmine leaf fractions

Bacteria	Fractions			Antibiotics	
	AE	NE	AQ	Vancomycin	Oxacillin
Gram-negative bacteria					
Vibrio cholerae	25	>50	>50		
Vibrio parahaemolyticus	0.78	1.56	>50	3.0	>256
Gram-positive bacteria					
Bordetella pertussis	>50	>50	>50	3.0	>256
Corynebacterium diphtheriae	>50	12.5	>50	1.0	0.75
Streptococcus milleri group	>50	>50	>50	4.0	0.38
Streptococcus sobrinus	12.5	25	>50	1.5	0.75
Streptococcus pneumoniae	>50	>50	>50	3.0	0.25
Staphylococcus Coagulase-negative	>50	>50	>50	0.09	2.0
Bacillus subtilis ATCC 26633	>50	>50	>50	6.0	>256
Streptococcus mutans ATCC 27175	>50	>50	>50	0.02	<0.02
Staphylococcus aureus ATCC 25923	1.56	0.78	>50	1.0	0.03
Drug-resistant strains					
Staphylococcus aureus MRSA N1	3.13	12.5	>50	2.0	>256
Staphylococcus aureus MRSA 20625	3.13	6.25	>50	1.5	>256
Staphylococcus aureus MRSA 20626	6.25	25	3.13	2.0	>256
Staphylococcus aureus MRSA 20627	0.39	1.56	6.25	2.0	>256
Staphylococcus aureus MRSA 20630	6.25	>50	>50	1.5	>256
Staphylococcus aureus MRSA 20631	6.25	12.5	>50	48	>256
Staphylococcus aureus MRSA 20633	0.36	25	>50	1.0	>256
Staphylococcus aureus MRSA 20636	0.78	3.13	>50	1.5	>256
Staphylococcus aureus VRSA 20622	3.13	>50	>50	>256	>256
Staphylococcus aureus VRSA 20623	0.78	3.13	>50	>256	>256
Staphylococcus aureus VRSA 20624	0.78	6.25	25	>256	>256
Staphylococcus aureus VRSA 21083	12.5	12.5	>50	>256	>256

4. Conclusions

Our research revealed that different fractions of Spanish jasmine exhibit varying degrees of total phenolic content and antioxidant activity. We observed that the AE fraction displayed the highest levels of both total phenolic content and antioxidant potency. Furthermore, the isolated compound oleuropein demonstrated remarkable antioxidant activity. Additionally, we found that the AE fraction exhibited impressive antibacterial capabilities, particularly against VRSA strains. Interestingly, it displayed higher activity levels than vancomycin and oxacillin. Notably, the AE fraction demonstrated the highest activity level against all tested VRSA strains resistant to both antibiotics. These findings highlight the potential of Spanish jasmine as a natural source of antioxidants and a potential medicinal herb for human use.

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