

Original article

Antimicrobial Activity Assessment of *Eriobotrya japonica* Leaf Extracts from Libya: An In Vitro Study

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Abstract

The increasing prevalence of antimicrobial resistance among bacterial and fungal pathogenic strains poses a significant global health challenge, emphasizing the urgent need for alternative therapies. The use of natural products derived from plants has gained attention as a potential solution. This study investigated the antimicrobial activity of the leaf extracts of the *Eriobotrya japonica* plant cultivated in different regions in Libya. Crude extracts were prepared using four solvents (water, 70% ethanol, chloroform, and hexane) and evaluated against four pathogenic bacterial strains (*Escherichia coli* ATCC[®] 25922, *Streptococcus pyogenes* ATCC[®]12344, *Klebsiella pneumoniae* ATCC[®]10031, *Staphylococcus aureus* ATCC[®]6538) and one fungal strain (*Candida albicans* ATCC[®]10231) using the disc diffusion method. The antimicrobial activity of *Eriobotrya japonica* varied with different solvent types. Ethanol extract showed the highest activity, with inhibition zones of 44.5 mm against *S. aureus*, 39 mm against *S. pyogenes*, 25 mm against *E. coli*, 23 mm against *K. pneumoniae*, and 20.6 mm against *C. albicans*. Chloroform extract exhibited moderate activity, hexane showed lower effectiveness, and the aqueous extract displayed minimal or no activity. These findings emphasize the critical role of solvent polarity in extracting bioactive compounds, with ethanol and chloroform being the most effective for *E. japonica*. Gram-positive bacteria were generally more susceptible than Gram-negative bacteria. To the best of our knowledge, this study represents the first report of the broad-spectrum antimicrobial activity of these plant extracts grown in Libya, suggesting their promising application in new drug discovery and pharmaceutical fields.

Keywords. *Eriobotrya japonica*, Medicinal Plant Extracts, Antimicrobial Resistance, Phytochemical Screening.

Introduction

The increasing pathogenic microbial resistance to antibiotics, as well as their side effects and high expense, has led to a search for new infection-fighting strategies to control microbial infections. Generally, naturally produced compounds, if compared with synthetic and semi-synthetic antibiotics, have better potential, fewer side effects, and minimal toxicity [1]. Synthetic antibiotics are no longer the safest agents used in the treatment of diseases because of their high cost, side effects, and microbial resistance, which has developed in multiple forms by opportunistic infectious microbes [2].

Medicinal plants are a significant source of chemicals with therapeutic value. Medicinal herbs have been utilized for centuries to treat a variety of illnesses. These plants can yield a range of bioactive compounds (phytochemicals) for the creation of more advanced medicinal products when analyzed systemically. Recent years have seen an increase in interest in the pharmacological analysis of diverse plants utilized in various traditional medical systems [3]. Using natural antibiotics, which are of plant origin, does not induce secondary side effects (abdominal pain, diarrhea, cramping, headache, nausea, and vomiting, etc.). Plant products may be used as alternative antibiotics without causing bacterial resistance [4]. Plant-derived products contain a great diversity of phytochemicals such as steroids, phenolic acids, flavonoids, tannins, lignin, and other compounds [5]. These compounds possess numerous health-related effects such as antibacterial, antioxidant, antimutagenic, anticarcinogenic, and vasodilatory activities [6].

Eriobotrya japonica (EJ), commonly known as loquat, is a fruit tree from the Rosaceae family, traditionally used in various cultures for its medicinal properties. Various parts, like leaves, peels, and fruits, have been shown to possess various useful health benefits. The leaves are dark green, simple, alternate, 10–25 cm long, tough, and leathery in texture, with a serrated margin [7]. In Unani medicine, it is vastly utilized in many illnesses, like fevers, nausea, deranged sanguinous humour (diseases due to morbidity of blood), indigestion, liver diseases, vomiting, dysentery, wounds, inflammations, etc. Loquat plant contains many active constituents, such as glycosides, flavonoids, polyphenolic compounds, tannin, etc., and nutritional and mineral compounds like carotenoids, vitamins, starch, amino acids, sugar, and others. According to various pharmacological studies, it is found that the plant has many biological effects like antitussive, anti-melanogenic, anti-diabetic, anti-inflammatory, antimicrobial, kidney protective, hepatoprotective, and hypolipidemic activity. This review aims to shed light on the therapeutic applications of loquat based on both traditional Unani literature and scientific studies conducted on different parts of the plant [8,9].

Phytoconstituents in loquat, a broad range of phytochemical compounds like phenols, alkaloids, cardiac glycosides, flavonoids, mucilage, gums, and phytosterols, have been reported, which are responsible for various biological activities [8]. By using high-performance liquid chromatography, flavones like quercetin derivatives, hydroxycinnamic acid derivatives like chlorogenic acid, along with other p-coumaric acid and caffeic acid derivatives, have been identified in the leaves, fruit, and flowers of loquat [10].

Various extraction methods are used to extract the desired bioactive compounds from the plant materials, e.g., solvent extraction, distillation method, pressing, and sublimation. Solvent extraction is the most widely used extraction method when extracting from plant material [11].

The solvent selection is crucial in determining the bioactive compounds of plants used for extractions. Ideal extraction solvent properties include low toxicity, evaporating easily at low temperatures, having good solubility of the target compound, and being sufficiently volatile. The factors affecting the selection of solvents are the rate of extraction, diversity of compounds extracted, ease of handling of extracts, and the cost-effectiveness of the extraction solvents and targeted compounds. Plants consist of various bioactive compounds with varying polarities. Various techniques have been developed and used to obtain pure compounds and to determine their structure and biological activity. Many solvent extractions have been done to obtain phytochemical compounds for their activity against pathogens [11,12].

This study aims to evaluate the antimicrobial activity of leaf extracts from *Eriobotrya japonica* against certain microbial pathogens, with the goal of exploring a new source of natural antibiotics, providing insights into potential candidates for the future development of therapeutic drugs to treat some infectious diseases, and investigating their potential as sources of novel antimicrobial compounds.

Materials and Methods

Collection of plant samples

Healthy *Eriobotrya japonica* plants, including leaves, were collected during October 2025 from different geographical regions N 32° 6'18".407" and E 14° 3'12".302" from Al-Khums City, Libya, and identified according to the taxonomic characters at the Department of Botany, Faculty of Sciences, Elmergib University. A voucher specimen of the plant was deposited at the herbarium of the department. The plant samples were placed in plastic bags, stored in a refrigerator, and then used for extraction.

Plant Samples processing and drying plant material

Healthy plant leaves should be picked early in the morning because they are fresh and dew-free. Use clean scissors or shears to cut healthy leaves. Plant leaves were washed thoroughly with running tap water, followed by double-distilled water. Spread plant leaves in a single layer on a clean surface in a shaded, ventilated area. Avoid direct sunlight to retain their color and active compounds. Once dried, store the plant material in labeled, airtight containers away from moisture and light. Plant leaves are dried and powdered and then used for extraction [3] (Figure 1).



Figure 1. *Eriobotrya japonica* with leaves dried and powdered

Preparation of the different crude extracts from the plant

Extracts were prepared using the maceration (soaking) method as previously described by [11,13]. Weigh the powdered 20g of the powdered plants were mixed with 400 ml of polar selected solvents (water, ethanol 70%) for polar compounds, non-polar and semi-polar solvents (hexane and chloroform), for non-polar and semi-polar compounds in a ratio of 1:20 (w/v), depending on the solubility of the compounds. Place the mixture in a clean, sealed container (conical flask 500 ml) at room temperature for 48 hours, under continuous mixing until the biomass matter is dissolved (Figure 2 A). After maceration, strain the mixture through a muslin cloth or filter paper. Collect the filtrate (liquid extract) (Figure 2 B). To obtain a concentrated extract, evaporate the solvents from the filtrate using a rotary evaporator (IKA, RV10, Germany) under vacuum at 150 rpm and at a 45 °C water bath until complete dryness, adhering to the methodology outlined by Zhu et al. [14]. The dried crude extracts were dissolved in methanol for further studies [15,16].

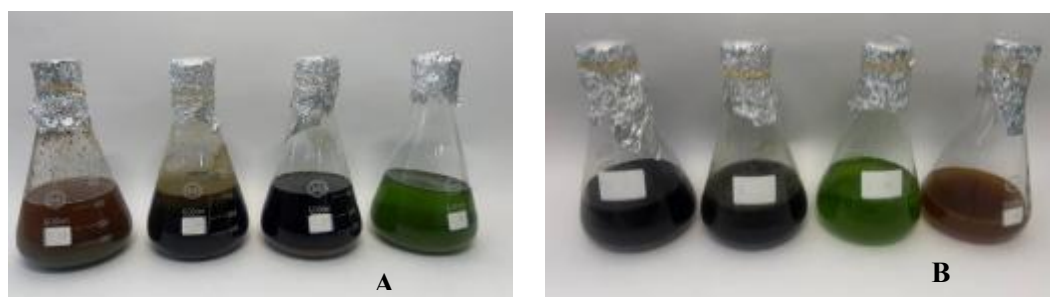


Figure 2. Extraction of bioactive compounds from *Eriobotrya japonica* leaves by organic and aqueous solvents. (A): Extracts after maceration. (B): Filtrates collected after filtration.

Investigation of the biological activity of the crude extracts

Tested organisms

The antimicrobial activity of *Eriobotrya japonica* crude extracts was tested using five pathogenic bacterial strains, *Escherichia coli* (ATCC[®] 25922), *Streptococcus pyogenes* (ATCC[®]12344), *Klebsiella pneumoniae* (ATCC[®]10031), *Staphylococcus aureus* (ATCC[®] 6538), and *Candida albicans* (ATCC[®]10231), which were obtained from the Microbiology Unit of the Central Laboratory at Al-Khoms Educational Hospital.

Preparation and standardization of inoculum

A few colonies from each of the tested isolates were first inoculated into test tubes of 5ml of sterile normal saline separately, and each of cultures turbidity were adjusted to 0.5 McFarland turbidity standard (prepared by addition of 1%w/v solution of barium chloride + 1 % v/v solution of sulphuric acid) for sensitivity tests as described by NCCLS and CLSI [17-19]. The comparison between two opacities of the bacterial suspension tube and 0.5 McFarland tube was done by placing the tubes against a black background [20].

Antimicrobial activity test of plant extracts (Agar disk diffusion assay)

Disk diffusion assay is the usual applicable method for assessing the antimicrobial susceptibility pattern in most institutions and hospitals [21]. Disc diffusion is an assay based essentially on the placing of filter paper discs, impregnated with antibiotic or crude extract, on the surface of agar immediately after inoculation [22]. This technique was originally standardized by Bauter et al. [23] and subsequently modified in the report of the World Health Organization [24].

Disk diffusion test was conducted to assess the antimicrobial activity of the pathogenic microbial strains and crude extracts. 100 µl of test inocula at a concentration of 10^8 CFU.mL⁻¹ were distributed using sterile cotton swabs onto Mueller-Hinton Agar media for the tested pathogenic bacterial strains and Sabouraud dextrose agar for the *C. albicans* strain. 100 µL of the plant's crude extracts were loaded onto filter paper discs (1.5 cm diameter). The discs were air-dried and transferred to the surface of the agar medium. The plates were left in a refrigerator for 2 hrs (to allow the extract diffusion) and then incubated at 37°C for 24 h. The appearance of the zone of inhibition (Zoi) around the discs was noted and measured. All antimicrobial tests were applied in triplicate, and the mean Zoi in mm was assessed [25].

Statistical analysis

All experiments were performed in triplicate. Statistical significance was determined using one-way analysis of variance (ANOVA) with SPSS software version 18. The differences among means were evaluated using the Least Significant Difference (LSD) post-hoc test at a significance level of $p \leq 0.05$.

Results

Screening of antimicrobial activity for *Eriobotrya japonica* extracts with different solvents.

This experiment was carried out to investigate the ability of *Eriobotrya japonica* extracts (water extract, ethanol 70% extract, chloroform extract, and hexane extract) to inhibit the tested pathogenic bacterial and yeast strains. The tested crude extracts displayed different capabilities in controlling the tested pathogenic bacterial and yeast strains.

Investigation of the biological activity

Antimicrobial activity of *E. japonica* crude extracts against tested pathogenic strains

The results obtained in this study showed that the antimicrobial activity of the crude extract of *E. japonica* using different solvents (water, ethanol, chloroform, and hexane) against five pathogenic microbial strains are presented in (Table 1) and (Figures 3 and 4). The antimicrobial efficiency was evaluated by measuring the diameter of the inhibition zone (mm). Larger inhibition zones indicate stronger antimicrobial activity. The different superscript letters (a, b, c) indicate statistically significant differences at $P \leq 0.05$ according to the LSD test.

The results showed that the ethanolic extract exhibited the highest antibacterial activity against *E. coli*, with an inhibition zone of 25 ± 0.06 mm, which was significantly different from the other solvents. The chloroform extract showed moderate activity with an inhibition zone of 22 ± 0.20 mm, followed by the hexane extract with 19 ± 0.06 mm. In contrast, the aqueous

extract showed no antibacterial activity (NA). This suggests that the bioactive compounds responsible for the antimicrobial effect against *E. coli* are likely moderately polar or non-polar compounds, which are more efficiently extracted using organic solvents such as ethanol and chloroform. Overall, *E. coli* showed moderate sensitivity to the plant extract, which may be attributed to its Gram-negative cell wall structure, known to limit the penetration of antimicrobial compounds.

Among the tested microorganisms, *S. pyogenes* exhibited the highest sensitivity to the plant extract. The ethanolic extract produced the strongest inhibitory effect with an inhibition zone of 39 ± 0.06 mm, showing a significant difference compared with the other solvents. This was followed by the hexane extract with an inhibition zone of 33 ± 0.10 mm, and the chloroform extract with 29 ± 0.10 mm, while the aqueous extract showed the lowest activity with 24 ± 0.06 mm. These findings suggest that the bioactive compounds present in the plant extract are highly effective against Gram-positive bacteria such as *S. pyogenes*. This may be due to the simpler cell wall structure of Gram-positive bacteria, which allows easier penetration of antimicrobial phytochemicals.

K. pneumoniae showed a pattern similar to *E. coli*, where the ethanolic extract demonstrated the highest antibacterial activity, with an inhibition zone of 23 ± 0.12 mm, followed by the chloroform extract, 21 ± 0.00 mm, and the hexane extract, 17 ± 0.06 mm. The aqueous extract showed no inhibitory activity (NA). These results indicate that *K. pneumoniae* is less sensitive to the plant extract compared with *S. pyogenes*. This may also be explained by the Gram-negative nature of the bacterium, which possesses an outer membrane that acts as a barrier against many antimicrobial agents.

Staphylococcus aureus showed the highest sensitivity among all tested bacteria, with inhibition zones of 44.5 ± 0.00 mm by the ethanol extract and 36.1 ± 0.10 mm by the chloroform extract, while both aqueous and hexane extracts showed no activity. This indicates strong susceptibility to polar and semi-polar compounds. *Candida albicans* showed moderate sensitivity, with inhibition zones of 22 ± 0.05 mm by the hexane extract and 20.6 ± 1.154 mm by the ethanol extract, while both aqueous and Chloroform extracts showed no activity. This suggests that moderately nonpolar compounds may be responsible for the antifungal activity.

Table 1. Mean values of inhibition zones (mm) $\pm$ standard deviation (SD) recorded from different tested microbial strains after treatment by *E. japonica* crude extract in different solvents

Pathogenic strains	Solvents	Inhibition Zone (mm) (mean $\pm$ S.D)	Standard error (SE)
<i>E. coli</i> ATCC® 25922	Water	NA	NA
	Ethanol	25 ± 0.06^a	0.03
	Chloroform	22 ± 0.20^b	0.12
	Hexane	19 ± 0.06^c	0.03
<i>S. pyogenes</i> ATCC® 12344	Water	24 ± 0.06^c	0.03
	Ethanol	39 ± 0.06^a	0.03
	Chloroform	29 ± 0.10^b	0.06
	Hexane	33 ± 0.10^{ab}	0.06
<i>K. pneumoniae</i> ATCC® 10031	Water	NA	NA
	Ethanol	23 ± 0.12^a	0.07
	Chloroform	21 ± 0.00^b	0.00
	Hexane	17 ± 0.06^c	0.03
<i>S. aureus</i> ATCC® 6538	Water	NA	NA
	Ethanol	44.5 ± 0.00^a	0.00
	Chloroform	36.1 ± 0.10^b	0.05
	Hexane	NA	NA
<i>C. albicans</i> ATCC® 10231	Water	NA	NA
	Ethanol	20.6 ± 1.15^a	0.06
	Chloroform	NA	NA
	Hexane	22 ± 0.05^b	0.33

Data are shown as the mean $\pm$ SD of triplicate measurements from independent experiments. A-C means with different superscripts in the same column are considered statistically different (LSD test, $P \leq 0.05$).

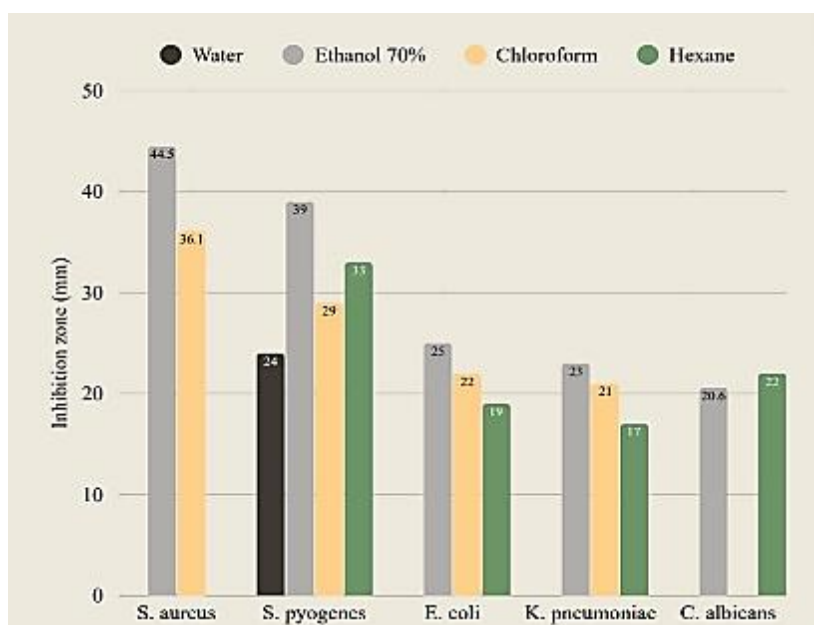


Figure 3. Antimicrobial activity of *E. japonica* extracts in different solvents.

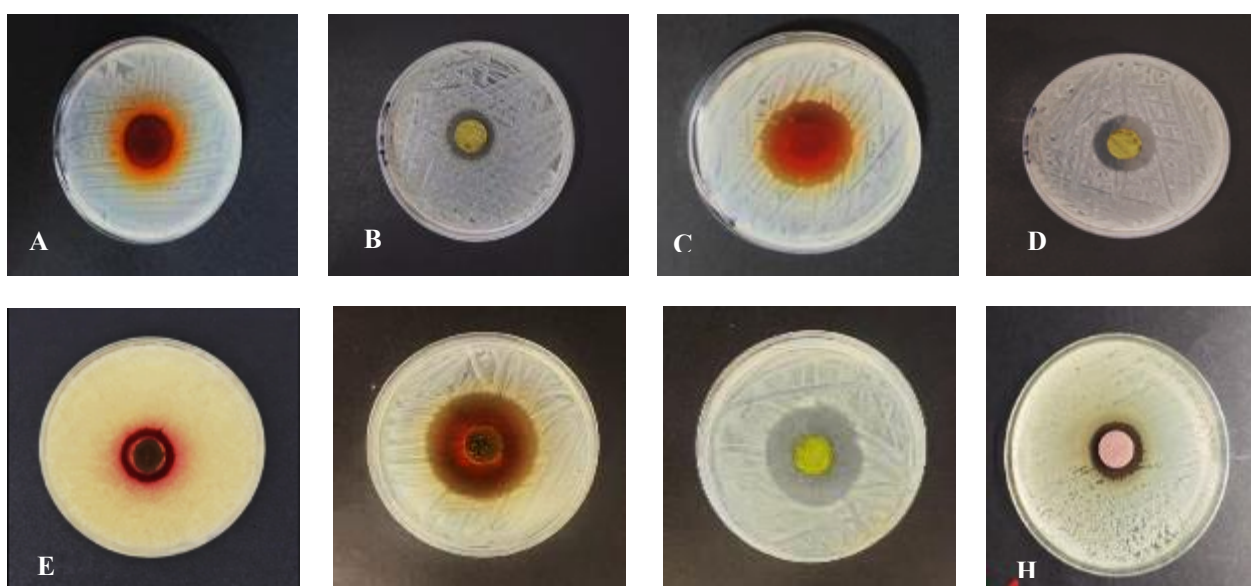


Figure 4 Antimicrobial susceptibility of *E. japonica* extracts against different microbial strains: A, B: Ethanol and Chloroform extracts against *E. coli*. C, D: Ethanol and Chloroform extracts against *S. pyogenes*. E: Ethanol extract against *K. pneumoniae*. F, G: Ethanol and Chloroform extracts against *S. aureus*. H: Ethanol extract against *C. albicans*.

Discussion

Bacterial resistance to currently used synthetic antibiotics is becoming a concern to public health. The development of bacterial super-resistant strains is resulting in currently used antibiotic agents failing to treat many bacterial infections. For this reason, many searches are ongoing for new antimicrobial agents, through the search of natural sources due to a perception that there is a lower incidence of adverse reactions of plant preparations compared to synthetic pharmaceuticals. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option [26].

Medicinal plants are a significant source of chemicals with therapeutic value. Medicinal herbs have been utilized for centuries to treat a variety of illnesses. These plants can yield a range of bioactive compounds for the creation of more advanced medicinal products when analyzed systemically. Recent years have seen an increase in interest in the pharmacological analysis of diverse plants utilized in various traditional medical systems. In recent decades, numerous traditionally recognized plants have been thoroughly examined using advanced scientific methods and reported to have a variety of therapeutic benefits, including antibacterial, antifungal, anticancer, and antioxidant properties [3].

The plant extracts are presented as promising sources for the search for new alternative substances, because they have a higher molecular diversity when compared to synthetic products chemically [27, 28]. The purpose of this work was to

investigate the antimicrobial activity of the Libyan plant, *Eriobotrya japonica*. *Eriobotrya japonica* (loquat), a broad range of phytochemical compounds like phenols, alkaloids, cardiac glycosides, flavonoids, mucilage, gums, and phytosterols have been reported [10]. To the best of our knowledge, this study represents the initial discovery of the bioactive compounds of the *Eriobotrya japonica* plant grown in areas in Libya and investigates their antimicrobial activity. Several studies reported that the crude extracts of *E. japonica* plants grown in cultivated areas in Japan, China, and India presented a broad antimicrobial spectrum [8].

The present study was designed to check the in vitro antimicrobial activity of water, ethanol 70%, chloroform, and hexane extracts of the plant (*Eriobotrya japonica*) leaves, cultivated in Libya. Extraction is the main step for recovering and isolating phytochemicals from tested plants, and its efficiency is affected by the chemical nature of phytochemicals, the extraction method, the sample particle size, and the solvent used [29]. Solvents such as water, ethanol 70%, chloroform, and hexane are commonly used for the extraction methods of medicinal plants. The study of bioactive compounds encompasses phytochemical and pharmacological approaches. Many plant parts contain bioactive components, e.g., bark, leaves, stems, fruits, and seeds. Phytochemicals are chemicals produced by the various parts of the plants namely, alkaloids, flavonoids, terpenoids, steroids, tannins, glycosides, etc. The bioactive compounds have various antimicrobial and antibacterial properties [11].

Bacterial infectious diseases represent an important cause of morbidity and mortality worldwide [1]. Therefore, the development of new antimicrobial agents for the treatment of bacterial infections is important. Our investigation shows the evaluation of the antibacterial activity of the tested plants against some pathogenic bacteria by the disc diffusion method. Our result indicated that most of the studied crude extracts were able to inhibit the growth of the five tested pathogenic microbial strains (*Escherichia coli* ATCC[®] 25922, *Streptococcus pyogenes* ATCC[®]12344, *Klebsiella pneumoniae* ATCC[®]10031, *Staphylococcus aureus* ATCC[®]6538, and *Candida albicans* ATCC[®]10231). Some plant extracts exhibited a good antibacterial activity, and some others exhibited a limited antibacterial activity; these activities may be due to the strong presence of different phytochemical compounds such as flavonoids, tannins, alkaloids, phenols, and saponins. The flavonoids and alkaloids have been found to possess antimicrobial and antioxidant properties in various studies [8]. The present study demonstrated that the crude extract of *E. japonica* exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as the tested fungal strain. The activity varied depending on the solvent used, highlighting the importance of solvent polarity in extracting bioactive compounds.

For *Escherichia coli*, the ethanol extract showed the highest activity with an inhibition zone of 25 mm, followed by chloroform 22 mm and hexane 19 mm, while the aqueous extract showed no activity. Similarly, *Klebsiella pneumoniae* exhibited moderate sensitivity, with inhibition zones of 23 mm, 21 mm, and 17 mm for ethanol, chloroform, and hexane extracts, respectively, while no activity was detected for the aqueous extract. In contrast, Gram-positive bacteria were more susceptible to the extract. *Streptococcus pyogenes* showed strong sensitivity, with inhibition zones of 24 mm for the aqueous extract, 39 mm for the ethanol extract, 29 mm for the chloroform extract, and 33 mm for the hexane extract, respectively. The highest antimicrobial activity in the entire study was observed against *Staphylococcus aureus*, where the ethanol extract produced an inhibition zone of 44.5 mm, followed by chloroform (36 mm), while both the aqueous and hexane extracts showed no activity.

Regarding antifungal activity, *Candida albicans* showed moderate sensitivity to the extracts, with inhibition zones of 22 mm for hexane and 20.6 mm for ethanol, while no activity was observed for chloroform and aqueous extracts. Overall, ethanol extract demonstrated the highest antimicrobial activity across most tested microorganisms, indicating its efficiency in extracting active compounds. The lower activity observed in Gram-negative bacteria compared to Gram-positive ones may be attributed to the presence of an outer membrane that restricts the penetration of antimicrobial agents. Additionally, the moderate antifungal activity observed in the chloroform extract suggests the presence of less polar bioactive compounds contributing to antifungal effects.

Our results are supported by the study of Cornejal *et al.* [30], in which they reported that the ethanolic extract obtained from dried leaves and stems of *E. japonica* showed significant inhibition of bacterial growth of *E. coli*. Previous studies have demonstrated that ethanolic and methanolic extracts (80%) of the stems of loquat extracted by the maceration process demonstrated an antimicrobial effect against bacterial and fungal strains linked to the presence of triterpenes and flavonoids. It was found to be more effective against *Candida albicans*, indicating that it can be used to treat fungal infections and has no effect on other strains of bacteria or fungi. The presence of flavonoids, tannins, and kaempferol 3-O- β -glucoside in the ethanolic and methanolic extracts of loquat stems is responsible for its strong antibacterial and antioxidant properties [8]. Several authors point out that there is a greater antimicrobial activity in Gram-positive bacteria such as *S. aureus*, compared to Gram-negative bacteria such as *E. coli* and *Salmonella*, due to the composition of the cell wall, which has a thick layer of lipopolysaccharides that acts as a hydrophobic barrier, which prevents the penetration of compounds, together with the presence of enzymes in the periplasmic space that limits the entry of substances from outside [31,32].

Conclusion

In this work, an investigation was performed to study the antimicrobial activity of leaf extracts from *Eriobotrya japonica* cultivated in Libya against certain microbial pathogens, with the goal of exploring a new source of natural antibiotics, providing insights into potential candidates for the future development of therapeutic drugs to treat some infectious

diseases, and investigating their potential as sources of novel antimicrobial compounds. The results of this study demonstrated that the extracts of *Eriobotrya japonica* possess significant antimicrobial activity, which varies depending on the solvent used. Among all extracts, ethanol exhibited the highest effectiveness against most tested microorganisms, while chloroform showed moderate activity, particularly against the fungal strain. In contrast, hexane and aqueous extracts showed little to no activity in most cases. Gram-positive bacteria were generally more sensitive than Gram-negative bacteria. Based on the results of this study, extracts of *Eriobotrya japonica* have promising potential as supplements or alternatives to antibiotics in combating drug-resistant bacteria, warranting consideration in future therapeutic strategies. Furthermore, future studies should determine the Minimum Inhibitory Concentration (MIC) of these plant extracts against various pathogens to establish the most effective concentrations for antimicrobial activity.

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Ethical approval: Not applicable

Research involving Human Participation and/or Animals: Not applicable

Competing interests: The authors declare no conflict of interest

Authors 'contributions: **SJA** suggested the research point, article investigation, designing experiments, conducting the experiments, recording the obtained results, data analysis, providing resources, preparing the original draft and contribution in manuscript writing, reviewing, and editing. **AMW** article investigation, designing and conducting experiments, data analysis, designing the statistical modeling, data representation, manuscript revising, and editing. All authors reviewed the article. **M.A.A, E.M.O, and A.A.T** contributed to the experimental work, laboratory investigation, and data collection. All authors reviewed and approved the final manuscript.

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