

Phytochemical Analysis of *Loranthus europaeus* Jacq Fruit Using FTIR, HS-SPME, and HPLC Methods, and Evaluation of the Antibacterial Activity of the Hydroalcoholic Extract of *Loranthus europaeus* Jacq Against *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*


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Article Info	ABSTRACT
Article type: Original Article	Objective: As the use of antibiotics continues to rise and resistance to them becomes more widespread, there has been a growing interest in natural treatments that may offer lower resistance and fewer side effects.
Article History: Received: Jan. 21, 2023 Received: Mar. 07, 2023 Accepted: Dec. 20, 2023 Published Online: May. 17, 2025	Methods: This study focused on analyzing the essential oil of <i>Loranthus europaeus</i> Jacq. fruit using various methods, including HS-SPME, GC-MS, FTIR, and HPLC. The antimicrobial effects of the fruit extract were tested against common bacterial pathogens like <i>Acinetobacter baumannii</i> , <i>Staphylococcus aureus</i> , and <i>Pseudomonas aeruginosa</i> . The fruits of <i>L. europaeus</i> were gathered from the mountains of Ilam, dried, and then used to prepare both essential oil and hydroalcoholic extracts. The chemical composition of these extracts was analyzed through Headspace Solid Phase Microextraction (HS-SPME), Gas Chromatography-Mass Spectrometry (GC-MS), and High-Performance Liquid Chromatography (HPLC).
 Correspondence to: Naser Abbasi	Results: The compound <i>phytol</i> was found to make up 16.25% of the hydroalcoholic extract. The IR spectrum revealed 27 distinct bands, indicating the presence of various chemical compounds with different stretching and bending vibrations. HPLC results identified rutin as the main compound in the hydroalcoholic extract, with a concentration of 223 µg/mL. In terms of antimicrobial activity, the extract exhibited an MIC of 20.62 µg/mL and an MBC of 330 µg/mL, though its effectiveness was lower compared to standard antibiotics like gentamicin and colistin.
Email: ilamfarma@gmail.com	Conclusion: The findings of this study suggest that <i>L. europaeus</i> contains a variety of chemical compounds that may have antimicrobial properties. While the antimicrobial activity of the extract was less potent than that of conventional antibiotics, it still shows promise as a natural alternative for combating bacterial infections. These results could pave the way for further research on the potential therapeutic use of medicinal plants in treating bacterial diseases.
	Keywords: Antibacterial, Phytochemistry, GC-SPME, HPLC, Rutin, <i>Loranthus europaeus</i> Jacq
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Introduction

Infectious

diseases remain one of the most significant challenges to public health, with widespread impacts on individual health

and healthcare systems [1]. These diseases are caused by various pathogens, including bacteria, viruses, fungi, and

parasites, and can lead to severe complications and mortality. Despite significant advancements in medical science, chronic and emerging diseases such as influenza, tuberculosis, and malaria continue to pose substantial threats to public health [2]. The emergence of drug-resistant microbes and the increasing resistance of bacteria to antibiotics have created considerable challenges in the treatment of these diseases. In this context, microbial infections caused by *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* represent examples of resistance-related issues that require the development of new therapeutic strategies [3].

Staphylococcus aureus is a gram-positive pathogen that typically inhabits the skin and mucous membranes of humans. Under certain conditions, it can cause a wide range of infections, including boils, abscesses, pneumonia, food poisoning, and endocarditis. The methicillin-resistant strain of this bacterium (MRSA) is a major challenge in treating infections due to its resistance to various classes of antibiotics [4, 5]. *S. aureus* has developed mechanisms to evade host immune responses and resist conventional treatments by producing enzymes and toxins such as coagulase, staphylokinase, and cytotoxins. These mechanisms complicate clinical management and necessitate the use of more specialized drugs and novel therapeutic strategies for controlling infections [6, 7].

Acinetobacter baumannii is an emerging drug-resistant pathogen, particularly problematic in hospital settings and among immunocompromised patients. This bacterium is known for its ability to resist a wide range of antibiotics, leading to severe infections such as pneumonia, sepsis, urinary tract infections, and surgical site infections. The resistance mechanisms of *A. baumannii*, including the production of β -lactamases, aminoglycosides, and other resistance enzymes, create significant treatment challenges [8, 9]. The spread of this bacterium in hospital environments and its transmission between patients have raised serious health concerns, highlighting the need for new preventive and therapeutic strategies [10, 11].

Pseudomonas aeruginosa is a gram-negative opportunistic pathogen, primarily responsible for severe infections in patients with weakened immune systems or those undergoing intensive treatments. This pathogen is associated with a variety of infections, including respiratory, urinary tract, wound, and bloodstream infections, as well as meningitis. *P. aeruginosa* is known for its high resistance to a broad spectrum of antibiotics, as well as its ability to produce various enzymes and toxins, such as fluorescent toxins and elastase. These characteristics complicate

treatment, making infections caused by *P. aeruginosa* particularly difficult to manage [12, 13]. The bacterium's ability to develop drug resistance and survive in hospital environments makes controlling and preventing its spread a major challenge in healthcare settings, requiring the use of specific antibiotics and targeted treatment strategies [14, 15].

The selection of appropriate antibiotics depends on factors such as the site and type of infection, the patient's characteristics, and the treatment objectives. In hospital infections, resistant bacteria are often present, and patient factors, such as age and immune status, significantly influence treatment decisions. The principles of antibiotic therapy emphasize using effective drugs with narrow activity spectra, minimizing side effects, and reducing microbial resistance [16]. Misuse of antibiotics, particularly in hospital settings, can contribute to the development of resistance, which is further facilitated by close contact and transmission between patients [17].

The use of medicinal plants and natural products to treat infectious and microbial diseases is of growing interest [18]. Many of these plants contain biologically active compounds such as alkaloids, flavonoids, and terpenes, which have proven antibacterial, antiviral, and antifungal properties [19, 20]. These compounds can serve as complementary treatments alongside chemical drugs by inhibiting the growth and proliferation of microorganisms or directly eliminating pathogens [21-23]. Additionally, medicinal plants are considered a promising therapeutic option due to their relatively low side effects compared to synthetic antibiotics and drugs, making them a sustainable and low-risk alternative for managing infections [24, 25].

L. europaeus is a parasitic plant that typically grows on trees such as oaks, cypress, and olives, drawing nutrients from its host [26]. This plant is a hemiparasite, using a structure known as a haustorium to absorb water and nutrients from its host. *Loranthus* spreads its seeds through wind or birds and thrives on older trees, particularly oaks. This plant is found in various regions, including southwestern Europe, Iran, and Iraq, with particular interest in its medicinal properties in the Ilam province of Iran [27, 28].

The aim of this study is to perform a phytochemical analysis of *L. europaeus* fruit using methods such as FTIR, HS-SPME, GC-MS, and HPLC, and to evaluate the antimicrobial activity of its hydroalcoholic extract against three clinically significant bacteria such as *S. aureus*, *A. baumannii*, and *P. aeruginosa*. This research seeks to explore the potential of *L. europaeus* as a natural alternative in combating resistant microbial infections.

Materials and Methods

Plant Collection

The fruits of *Loranthus europaeus* were collected from the Qalandar region in Ilam County, Iran. The collected samples were dried at room temperature and in the shade.

Extraction of Essential Oil and Compound

Analysis

The essential oil of *L. europaeus* was extracted using the Solid-Phase Microextraction [HS-SPME] method, followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. For this, 2 grams of the dried plant material were placed in a vial, and volatile compounds were absorbed by the SPME fiber. The compounds were then identified using a GC-MS system [29].

Instrument Specifications and Experimental Conditions

The experiments were performed using an Agilent 6890N Gas Chromatography system, equipped with an Agilent 5973 Mass Spectrometer. Nitrogen (99.999% purity) was used as the carrier gas, and the splitless injection method was applied for the analysis [29].

Phytochemical Extraction and Analysis

The plant extract was obtained using a Soxhlet extractor, with a mixture of ethanol, methanol, and water as solvents. The chemical compounds were identified using FTIR spectroscopy. Additionally, rutin in the *Loranthus* fruit extract was identified using High-Performance Liquid Chromatography (HPLC) [29].

Bacterial Strains Studied

This study focused on three clinically significant pathogenic bacteria *S. aureus*, *A. baumannii*, and *Pseudomonas aeruginosa*, all of which are known for their clinical importance.

Antibacterial Assays

The bacterial strains were tested for the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) using the microdilution method and microplate dilution, according to CLSI standards. The experiments were repeated three times to ensure accurate results. The results were compared with standard antibiotics, including methicillin, colistin, and gentamicin [29].

Results

In this study, essential oils and volatile compounds from the fruit of *L. europaeus* were analyzed using FTIR, HS-SPME, GC-MS, and HPLC for the quantification of rutin. The antimicrobial effects of the hydroalcoholic extract of *Loranthus* fruit were then assessed using the MIC and MBC methods.

The solid-phase microextraction (HS-SPME) method was used to extract volatile compounds, which were subsequently analyzed using GC-MS for phytochemical profiling. The results of the phytochemical analysis revealed that the essential oil of *L. europaeus* contained 39 chemical compounds. Among these compounds, the most prominent ones are listed in Tables 1 and 2. The chromatogram of the essential oil from *Loranthus* fruit is shown in Figure 1.

Table 1: Chemical Compounds in the Essential Oil of *L. europaeus* by GC-MS

Retention time	Compound	Area	%
12.018	Nonanal	1584113	2.22
13.578	Decamethyl- Cyclopentasiloxane	1896402	2.66
13.761	Menthone	1312246	1.84
14.533	Menthol	741286	1.04
15.19	Dodecane	6157873	8.64
15.487	Decanal	899351	1.26
16.345	Z-3-hexenyl isopentanoate	1402459	1.97
18.459	Menthyl acetate	1254801	1.76
18.557	Tridecane	1132143	1.59
19.482	Z-3-hexenyl tiglate	1845190	2.59
20.311	5-Methyltridecane	1987665	2.79
20.843	2-Methyltetradecane	1419120	1.99
21.031	Phytane	238545	0.33

21.106	Copaene	347245	0.49
21.666	Cyclotetradecane	2214485	3.11
21.774	Tetradecane	2128025	2.99
22.46	trans-Caryophyllene	3090256	4.34
22.877	Alloaromadendrene	384185	0.54
23.415	trans-Geranylacetone	3275037	4.59
23.557	Pentatriacontane	1353865	1.90
23.803	7-n-Hexyldocosane	792282	1.11
24.089	α -Amorphene	318274	0.45
24.26	α -Curcumene	220518	0.31
24.398	β -Ionone	3876598	5.44
24.615	pentadecane	1532505	2.15
25.369	δ -Cadinene	1119555	1.57
25.752	DIHYDROACTINIDIOLIDE	1524043	2.14
25.832	Methylundecane	1736614	2.44
25.986	5-Methylpentadecane	2439891	3.42
26.272	Tetratetracontane	678048	0.95
26.455	Farnesol	1137779	1.60
27.192	phytol	11585766	16.25
28.358	Dotriacontane	1653384	2.32
28.558	Lanol	568053	0.80
29.656	3-Cyclohexen-1-ol, 3-methyl-	2720461	3.82
31.37	Octadecane	1152459	1.62
31.485	Tritetracontane	952910	1.34
31.965	Tetrahydrogeranylacetone	1233409	1.73
33.153	Dibutyl phthalate	1374481	1.93

A total of 39 compounds were identified in the list, with Phytol accounting for the highest percentage at 16.25%. Dodecane, β -Ionone, trans-Geranylacetone, trans-Caryophyllene, Methylpentadecane, and Cyclotetradecane

follow in the ranking with percentages of 8.64%, 5.44%, 4.59%, 4.34%, 3.42%, and 3.11%, respectively. Figure 1 shows the chromatogram of the *Loranthus* essential oil.

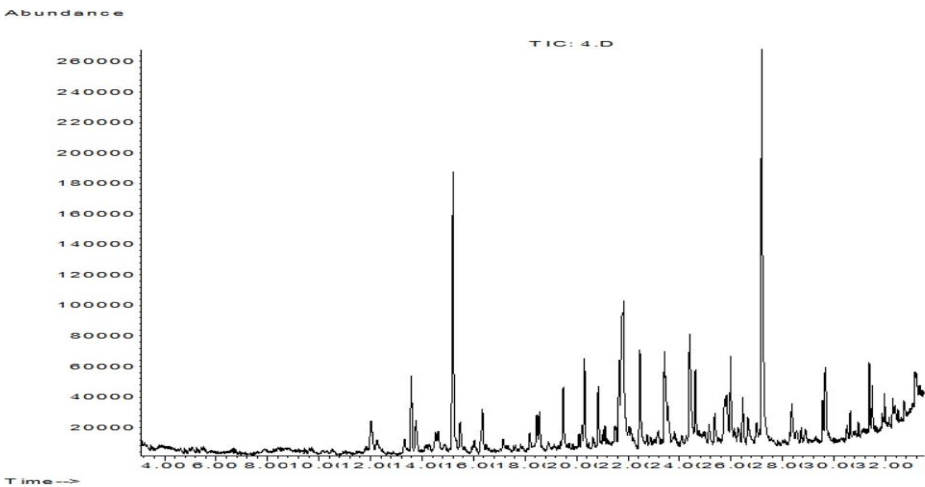


Figure 1. Chromatogram of *Loranthus* essential oil

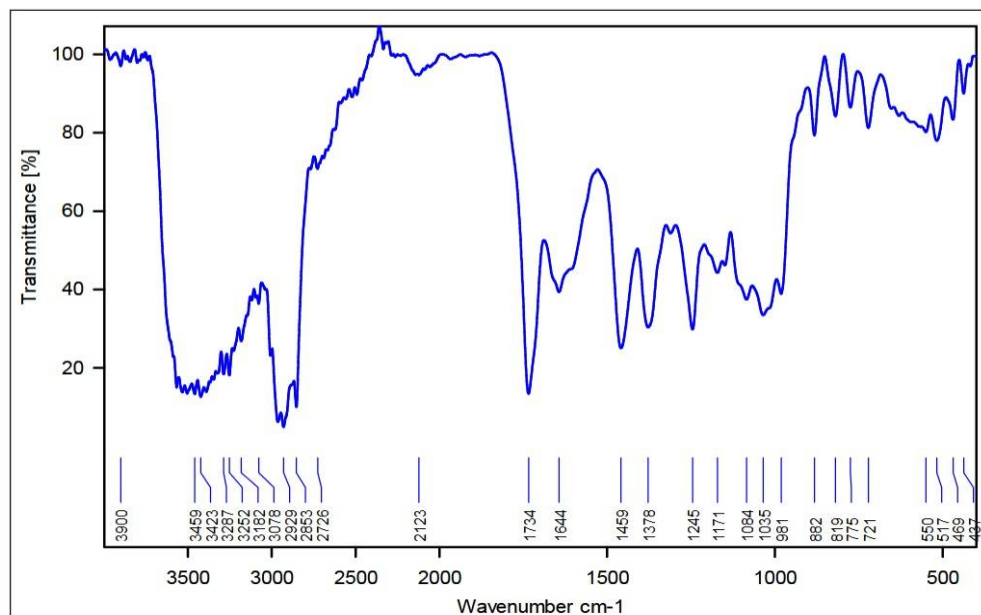


Figure 2. FTIR graph of Loranthus essential oil

Table 2. FTIR and functional groups of Loranthus fruit

Wavenumber [cm ⁻¹]	Functional Group + Vibration Type
3459	Stretching vibration of the OH group [Hydroxyl] in water or alcohols.
3423	Stretching vibration of the OH group in alcohols, acids, or water.
3287	Stretching vibration of NH in amines.
3252	Stretching vibration of NH in primary amines or amides.
3182	Associated with NH or hydrogen groups.
3078	Stretching vibration of the C-H group in aromatic compounds.
2929	Stretching vibration of C-H in alkanes.
2853	Similar to the previous frequency, associated with C-H in alkanes.
2726	C-H group in aldehydes or ketones.
2123	C≡C bond [Triple bond C-C] in alkynes.
1734	Stretching vibration of the C=O group in ketones and aldehydes.
1644	Stretching vibration of the C=O group in carboxylic acids or amides.
1459	Stretching vibration of C-H in alkanes.
1378	Stretching vibration of C-H in alkanes or aromatic compounds.
1245	Associated with C-O or C-N in aldehydes or amines.
1171	Associated with C-O in esters, ethers, or oxygen-containing groups.
1084	Associated with C-O in esters, ethers, or oxygen-containing groups.
1035	Usually related to C-N or C-O.
981	Typically related to CH ₂ in alkanes.
882	May refer to C-H bending in aromatic compounds.

819	Associated with C-H bending in aromatic compounds.
775	Associated with C-H bending in aromatic compounds.
721	Associated with C-H bending in aromatic compounds.
550	Associated with metal groups or M-O bonds.
517	Associated with metal groups or M-O bonds.
469	Refers to metal bonds or metal groups.
437	Refers to metal bonds or metal groups.

This table represents the infrared [IR] spectroscopy of Loranthus plant, examining molecular vibrations and various functional groups. Different frequencies in the IR spectrum correspond to stretching and bending vibrations of chemical bonds. Some key frequencies include:

3459 and 3423 cm^{-1} : OH stretching vibrations in water and alcohols. 3287 and 3252 cm^{-1} : NH stretching vibrations in amines and amides. 3078 cm^{-1} : C-H stretching vibrations in aromatic compounds. 2929 and 2853 cm^{-1} : C-H stretching vibrations in alkanes. 2726 cm^{-1} : C-H stretching in aldehydes and ketones 2123 cm^{-1} : C \equiv C bond in alkynes

1734 and 1644 cm^{-1} : C=O stretching vibrations in ketones, aldehydes, and carboxylic acids. 1459 and 1378 cm^{-1} : C-H bending in alkanes and aromatic compounds. 1245 and 1171 cm^{-1} : C-O or C-N stretching in aldehydes and esters

This IR spectrum is used to identify different functional groups such as OH, NH, C-H, C=O, and C \equiv C, helping to determine the chemical structure of the compounds. According to Figure 3, it is shown that rutin, the main compound in the hydroalcoholic extract of Loranthus fruit, is present at a concentration of 223 $\mu\text{g/mL}$.

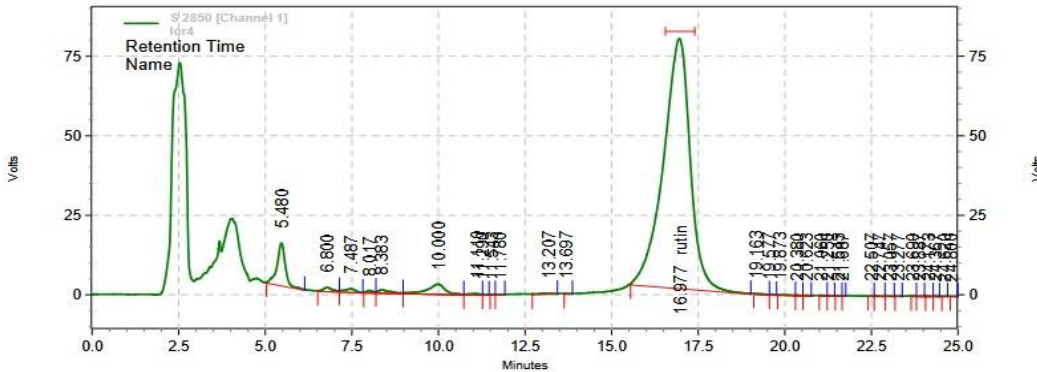


Figure 3: HPLC chromatogram of phenolic compounds in Loranthus europaeus fruit extract, including rutin

The microbroth dilution method was used to determine the MIC and MBC of Loranthus fruit extract. The results of the antimicrobial tests are presented in Table 3.

Table 3: Results of antimicrobial tests, MIC, and MBC of Loranthus plant and standard antibiotics

Bacterial Strain	[MIC ($\mu\text{g/mL}$)	[MBC ($\mu\text{g/mL}$)
<i>Staphylococcus aureus</i>	165	330
<i>Acinetobacter baumannii</i>	165	640
<i>Pseudomonas aeruginosa</i>	165	165
Hydroalcoholic Extract of <i>Loranthus europaeus</i>	20.62	330
Methicillin	2	64
Colistin	1	64
Gentamicin	0.5	64

In this study, the antimicrobial effects of several bacterial strains and different drugs were evaluated based on two indices: MIC [Minimum Inhibitory Concentration] and MBC [Minimum Bactericidal Concentration]. The results are as follows:

The antimicrobial testing results showed significant differences in the sensitivity of various bacterial strains to the drugs and extracts. The *S. aureus* strain had an MIC of 165 µg/mL and an MBC of 330 µg/mL, indicating relative resistance to antimicrobial treatments. The *A. baumannii* strain also had an MIC of 165 µg/mL and an MBC of 640 µg/mL, demonstrating higher resistance to treatment. The *P. aeruginosa* strain showed similar sensitivity to antimicrobial drugs, with both MIC and MBC values of 165 µg/mL. The hydroalcoholic extract of *Loranthus* fruit had an MIC of 20.62 µg/mL and an MBC of 330 µg/mL, indicating that the extract can inhibit growth and eliminate some bacterial strains, though its effect is less potent than that of chemical drugs. Among the chemical drugs, methicillin was effective for sensitive strains, with an MIC of 2 µg/mL and an MBC of 64 µg/mL. Colistin, with an MIC of 1 µg/mL and an MBC of 64 µg/mL, is recognized as an effective antimicrobial drug with a low MIC and appropriate MBC. Gentamicin, with an MIC of 0.5 µg/mL and an MBC of 64 µg/mL, demonstrated strong antibacterial inhibition and killing at very low concentrations. Overall, gentamicin and colistin were identified as the most effective antimicrobial agents, while the hydroalcoholic extract of *Loranthus* fruit, though inhibitory, was not as potent as the chemical drugs.

Discussion

The use of medicinal plants in the treatment of various diseases has gained significant attention from researchers today. In this regard, certain plant compounds are utilized for different disorders and diseases. Phenolic compounds, particularly flavonoids, are considered important bioactive substances due to their antioxidant effects, and have long been regarded for disease prevention and human health [30,31].

The results of this study showed that the leaf extract of *L. europaeus* mainly contains rutin, with MIC and MBC values for the *Staphylococcus aureus* strain being 6 and 196 µg/mL, respectively. The extract showed no significant antibacterial effect on *Acinetobacter baumannii* and *Pseudomonas aeruginosa* [32]. The essential oil of *L. europaeus* leaves had no antibacterial effect. Overall, the leaf extract of *L. europaeus* may be considered as a treatment option for infections caused by *S. aureus* [33]. The fruit extracts of *L. europaeus*, particularly the volatile oils they contain, might act as immunomodulators in bacterial infections. These

compounds contain chemicals that serve as chemotactic agents for neutrophils, stimulating and enhancing macrophage activity, which ultimately strengthens the body's immune response to bacterial infections [35]. Several studies have shown that the alcoholic extract of *L. europaeus* has an inhibitory effect on methicillin-resistant *Staphylococcus aureus*. In this study, a concentration of 20 mg/mL of the extract caused an inhibition zone of 17.28 mm in diameter. Additionally, concentrations of 100, 50, and 25 mg/mL resulted in inhibition zones of 13.28, 10.57, and 8.42 mm, respectively [36]. Analysis of the essential oil of *L. europaeus* showed that its major components include hexadecanoic acid and 1-eicosanol. Previous research has also shown that essential oils from *Solanum sisymbriifolium*, which contain the same compounds (hexadecanoic acid and 1-eicosanol), exhibit effective antibacterial activity against *S. aureus*. This antibacterial effect was observed at concentrations of 60 and 80 µg/mL for the plant's fruits and flowers, respectively [37]. A study on 24 rabbits examined the effect of the *L. europaeus* seed oil extract on pyogenic inflammation in *S. aureus*-infected wounds. The results showed that hyperemia and discharge increased in the early days and then decreased. Microscopically, infiltration of neutrophils and macrophages at the wound site was observed. High production of pro-inflammatory cytokines, such as IL-1 and IL-6, during bacterial infection was also noted, which helps in the chemotaxis of neutrophils and the clearance of bacteria and necrotic tissue [38-41].

L. europaeus contains bioactive compounds such as flavonoids, alkaloids, glycosides, carbohydrates, phenolic acids, and quercetin. These compounds play a crucial role in the plant's medicinal properties and can have various effects on human health [42-45]. Since iron (Fe^{2+}) is essential for the growth of organisms inside macrophages, the chelation effects of quercetin on iron metabolism in parasites were studied, and its leishmanicidal effects were proven. Quercetin forms chelate complexes with iron, limiting parasite access to iron and disrupting their growth and proliferation. Additionally, quercetin can stimulate the production of reactive oxygen species [ROS], which leads to mitochondrial dysfunction and parasite death. These features make quercetin a promising therapeutic compound for leishmaniasis [46]. A study showed that the ethanolic and methanolic extracts of *Loranthus europaeus* at concentrations ranging from 50 to 200 µg/mL exhibited significant effects on bacteria and fungi. Moreover, the anti-AChE (acetylcholinesterase) activity for the ethanolic and methanolic extracts was measured at 13.51 ± 0.81 and 22.79 ± 1.86 µg/mL, respectively, while the anti-BChE (butyrylcholinesterase) activity was measured at

27.84±0.62 and 33.08±1.63 µg/mL, respectively. These properties indicate that *L. europaeus* can be an effective natural substance in the design of new drugs for various therapeutic areas [47]. A study showed that monoterpenes in *L. europaeus* seed oil extract are responsible for the plant's antioxidant effects. Additionally, another study indicated that pure antioxidants like gallic acid, caffeic acid, and quercetin also contribute to these effects [48]. These compounds prevent oxidative damage by reducing free radical production and enhancing the body's defense system, thus playing a role in the prevention of diseases related to oxidative stress [49]. It has been shown that the extract of *L. europaeus* also has effects against *Helicobacter pylori* [50]. The methanolic extract of *Loranthus micranthus*, rich in phytochemicals and exhibiting high antioxidant and antibacterial activities against *S. aureus*, shows great potential for use in treating bacterial infections and diseases related to oxidative stress [51]. Rutin is a flavonoid glycoside found in plants like *L. europaeus*, acting as both a metabolite and an antioxidant [52]. This compound is a disaccharide derivative of quercetin glycoside [53]. Rutin has demonstrated therapeutic, antibacterial, antimalarial, antiviral, and antifungal effects, and exhibits effective antibacterial activity against bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Shigella sonnei*, *Klebsiella*, and *Bacillus subtilis* [54-56]. The bioactive compounds of medicinal plants, with diverse biological effects such as antibacterial, anti-inflammatory, and antioxidant properties, play a crucial role in disease prevention and treatment [57,58].

Conclusion

The results of studies indicate that *Loranthus europaeus* contains various bioactive compounds that exhibit different therapeutic effects, including antibacterial, antioxidant, and anti-inflammatory activities. The leaf extract of this plant is particularly effective against *S. aureus* and could be considered as a treatment option for infections caused by this bacterium. Additionally, compounds such as quercetin and rutin play significant roles in preventing oxidative stress-related diseases and bacterial infections. Overall, this plant has the potential for use in the design of new drugs with various therapeutic properties.

Statements and Declarations

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Competing interests:

The authors have no competing interests to declare that are relevant to the content of this article.

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