Research Article

Phytochemical Screening And Antibacterial Activity From Iraqi Euonymus Japonicus L.Root Extract

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Abstract

Euonymus japonicus a member of the celastraceous family, is utilized both as an ornamental plant and in traditional Chinese medicine. The limited knowledge regarding the primary active compounds and potential biological effects of Iraqi Euonymus japonicus roots prompted the initiation of this in vitro investigation. The chemical composition was determined through High Performance Liquid Chromatography (HPLC). Additionally, the antibacterial properties were assessed using the agar well diffusion method against three pathogenic bacteria. The findings revealed that the key active compounds present in the ethyl acetate fraction of Euonymus japonicus roots included naringenin, kaempferol, vitexin, and quercetin. Notably, the ethyl acetate extract exhibited significant antimicrobial effectiveness at concentrations of 50, 100, and 200 µg/ml, with inhibition zones measuring 15-23 mm for *Streptococcus pneumoniae, 10-20 mm for Morganella morganii, and 7-13 mm for Actinomyces. These results were compared to the positive control cefixime, which showed inhibition zones ranging from 0 to 23 mm.

Key word: Euonymus japonicus**,** roots, antibacterial, HPLC.

Introduction

For centuries, nature has acted as a substantial source of medicinal herbs, with a significant number of contemporary pharmaceuticals originating from natural resources, particularly plants (1). Historically, medicinal plants have served as the cornerstone for treating various ailments, whether in powdered form, liquids, or mixtures. Over the past 20 to 25 years, there has been an increase in the use of plants as complementary and alternative medicine (2). The World Health Organization reports that 65-80% of the global population relies on traditional medicines for basic healthcare (WHO). Moreover, the emergence of multiple drug-resistant bacterial strains due to the widespread use of medications for infectious diseases has sparked renewed interest in herbal remedies(3). Numerous herbs, which have been used for flavoring in foods and beverages throughout history, have also been recognized for their health benefits, including preventing spoilage and acting as antimicrobials against harmful microorganisms. Across the country, many medicinal plants are undergoing extensive research to explore their antibacterial properties (4)(5)(6) , However, structured research remains limited. In the absence of scientific validation regarding their efficacy, the credibility of these treatments is often questioned, leading to restrictions on their usage in certain areas. Ongoing phytochemical and medicinal studies on a variety of plants have already led to the discovery of several natural antibiotics (7).

Euonymus japonicus**,** a member of the Celastraceae family, was utilized in the current research to evaluate its antibacterial properties. This species is cultivated in Iraq (Figure 1), Euonymus japonicus has traditionally served as an ornamental plant in gardens as well as in traditional Chinese medicine for treating various ailments, particularly those related to liver health. The bark possesses diuretic, tonic, and antirheumatic qualities, while the cooked leaves are thought to assist during challenging childbirths. To date, there has been limited research on Euonymus japonicus**,** which has led to increased interest in conducting scientific investigations into the polyphenolic content and antibacterial efficacy of ethyl acetate extracts from the roots of Iraqi Euonymus japonicus

Figure (1): photo of Euonymus japonicus L.

2. Material and methods

2.1 Chemicals and Apparatus:

All biomolecules and organic solvents utilized in this study are sourced from Scharlab S.L. in Spain, ensuring effective quality management, with the exception of acetonitrile and methanol, which are of HPLC grade and obtained from Sigma-Aldrich in Germany. Standards for naringenin, vitexin, quercetin, and kaempferol were supplied by Chengdu Biopurify Phytochemicals in China. The research employed High-Performance Liquid Chromatography (HPLC) using a Knauer Germany 10AV-LC system, along with a rotary evaporator (BCHI Rotavapor R-205 from Switzerland) and a sonicator (Baranson Sonifier from the USA)

2.2 Plant sample Collection and Identification:

The plant material of Euonymus japonicus L. consisted of fresh roots that were cultivated in Iraq and collected from Zayona plant nurseries in Baghdad between October and November 2021. Identification and verification of the species were conducted by Dr. Israa Abdulrazaq, a taxonomist at the Biology Department of the College of Science, University of Baghdad. Prior to processing, the plants were dried in a shaded area at room temperature before being ground and weighed with an electric mixer.

2.3. Extraction of Euonymus japonicus

25 grams of the powdered plant material were subjected to maceration in hexane for seven days, with regular shaking at room temperature. Following this, the extract was filtered, and this defatting process was repeated three times. The plant material was then allowed to dry before undergoing extraction using a hot extraction method through Soxhlet apparatus with 85% ethanol as the solvent. The methanolic extract was filtered, and the solvent was removed under reduced pressure using a rotary vacuum evaporator at a temperature not exceeding 40°C, resulting in a dark green residue referred to as crude extract. To partition the crude extract, ethyl acetate (3 x 200 ml) was employed; it was subsequently dried over anhydrous sodium sulfate, filtered, vacuum-dried, weighed, and prepared for qualitative and quantitative analyses as well as biological assessments. (8)· As illustrated in figure 2.

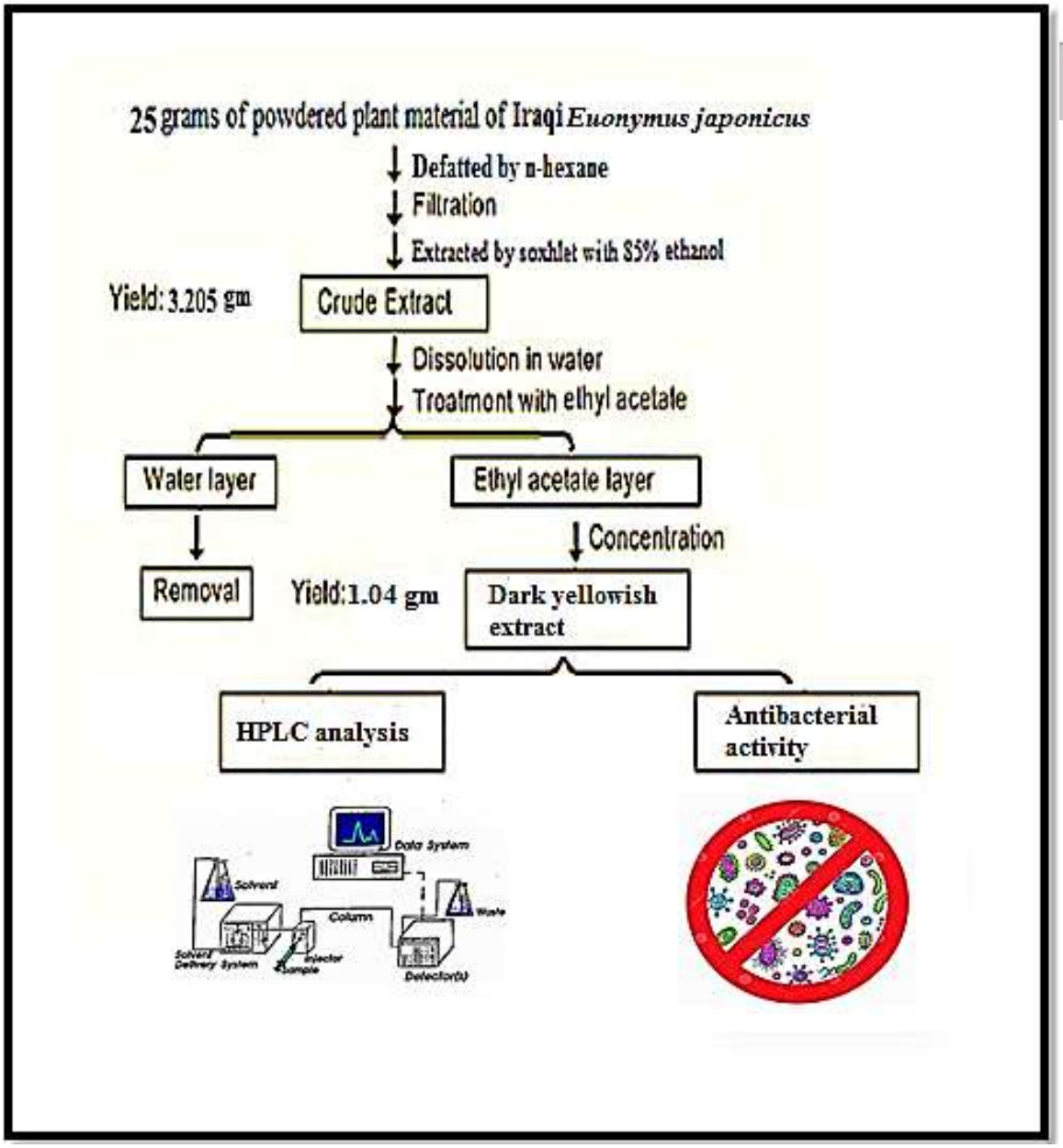


Figure (2): Illustration graphic for extraction procedure of Iraqi Euonymus japonicus L roots.

2.4. Qualitative and quantitative estimation of polyphenolic compounds by RP-HPLC:

An efficient, straightforward, and rapid RP-HPLC method can reveal diverse compositions of plant extracts. This technique is also employed for both qualitative and quantitative assessment of extraction content, including the calibration (using linear least squares regression) of analyzed components against established reference standards, all under consistent chromatographic conditions by aligning retention times and UV spectrum profiles. In this study, this method was applied to quantify the ethyl acetate fraction derived from the root parts of Euonymus japonicus.

2.4.1 Preparations of standard and samples for analysis

Four reference standards of polyphenol and dried roots in an ethyl acetate fraction were prepared for RP-HPLC analysis. The samples were dissolved in methanol and subjected to sonication for 30 minutes at 25°C with a 60% duty cycle, followed by centrifugation at 3000 rpm for 15 minutes. After drying the

sample solution, the residues were each suspended in 1 ml of HPLC-grade methanol, homogenized, and filtered through a 0.45 mm membrane filter. The resulting solutions were stored at 4°C for subsequent analysis. For the HPLC analysis, 20 µL of each sample and standard was loaded into the equipment. (9)

2.4.2- RP-HPLC conditions for qualitative and quantitative analysis

Using a Knauer Germany 10AV-HPLC system, an analysis was conducted on the ethyl acetate fraction extracted from the roots of Euonymus japonicus, along with four different concentrations of each reference standard. The assessment was performed at a wavelength of 280 nm utilizing an RP-C18-ODS column (250 mm x 4.6 mm particle size), which was maintained at room temperature. The mobile phase consisted of solvent A (1% acetic acid in HPLC-grade water) and solvent B (acetonitrile). The elution process followed a descending mode with a flow rate set at 1 ml/min. The gradient was as follows: 60% B from 40 to 50 minutes, shifting to 10% A and 90% B from 50 to 55 minutes, then returning to 90% A and 10% B for subsequent intervals—specifically maintaining this ratio for several subsequent cycles. Each standard and sample injection volume was fixed at 50 µL. (10)

2.5 evaluation of antibacterial activity

2.5.1 identifecation of antibacterial activity by agar well diffusion method

The Mueller-Hinton/Nutrient agar plates had been inoculated with 0.2 ml of the starter culture. Holes (7.5 mm in diameter were bored out of agar media using a cleansed stainless-steel borer and refilled with 0.2 cc of the extracted. Dishes contaminated with various bacteria were maintained for 24 hrs. at 37°C first before size of any resulting inhibition zone was determined. The study was repeated two times for extract and bacterial strain combination. Microorganisms with such a defined inhibition zone greater than 10 mm were designated as responsive. The antibacterial effect of phytoconstituents at various concentrations was matched with that of commonly used antibiotics, including cefixime (5 g/disc).Test organisms

Two groups of bacteria (two Gram positive: Actinomyces naeslundii, Streptococcus pneumonia, one Gram negative: Morganella morganii) were obtained from the almustansiriya university /college of pharmacy.

2.6.2. Preparation of sample solution

To evaluate the bactericidal efficacy, 0.1 g of the ethyl acetate fraction was individually dissolved in 5 mL of DMSO. This solution was subsequently diluted to create three different concentrations: 200 mg/mL, 100 mg/mL, and 50 mg/mL. The study focused on examining the antimicrobial effects of the ethyl acetate fraction derived from the roots of Euonymus japonicus L. against bacterial strains responsible for infections. All bacterial strains were grown on Mueller Hinton agar (MHA) at a temperature of 37°C for a duration of 24 hours. The antibacterial properties of the extracts were then assessed utilizing the Agar well diffusion method.(12)(13). For the preparation of petri dishes, sterile Mueller-Hinton agar (MHA) was employed as the growth medium. The test strains were applied to the surface of the solidified medium and allowed to dry at room temperature for 10 minutes. Subsequently, a sterilized cork borer was used to create two wells, each measuring 5 mm in diameter, in the center of the petri dish. After a diffusion period of 12-15 minutes at room temperature, the inoculated plates were incubated at 37°C for a duration of 24 hours. The evaluation of bactericidal activity was conducted after incubation by measuring the inhibition zone in millimeters (mm). The antibacterial efficacy of plant extracts was tested against three microorganisms: Actinomyces naeslundii, Streptococcus pneumoniae, and Morganella morganii, which is gram-negative. Dimethyl sulfoxide (DMSO) served as the negative control, while cefixime acted as the positive control.(14)(15)(16)

3. Result and Discussion

Based on the processes of defatting, extraction, and fractionation using various solvents, the extracts obtained from the roots of Euonymus japonicus exhibited different yield percentages. The quantities and percentage yields of extracts in n-hexane, methanol, and ethyl acetate were measured and are detailed in Table 1.

Table (1): the quantities and percentage yield of the extracts with n-hexane, methanol and ethyl acetate:

Fraction of plant extract	Quantity	Percentage yield
n-hexane	5gm	0.05%
Methanol	3gm	0.03%
Ethyl acetate	1gm	0.01%

3.1 RP-HPLC analysis:

HPLC with C18 columns is the predominant method employed for the analysis of polyphenols present in a variety of food items and plant extracts. The findings from the RP-HPLC chromatogram indicate that this approach effectively determines both the qualitative and quantitative levels of polyphenols found in the ethyl-acetate fraction derived from Iraqi Euonymus japonicus roots. (17), When the retention times of both reference standards and four phytoconstituents found in the ethyl acetate fraction were similar, as shown in Figure 3.

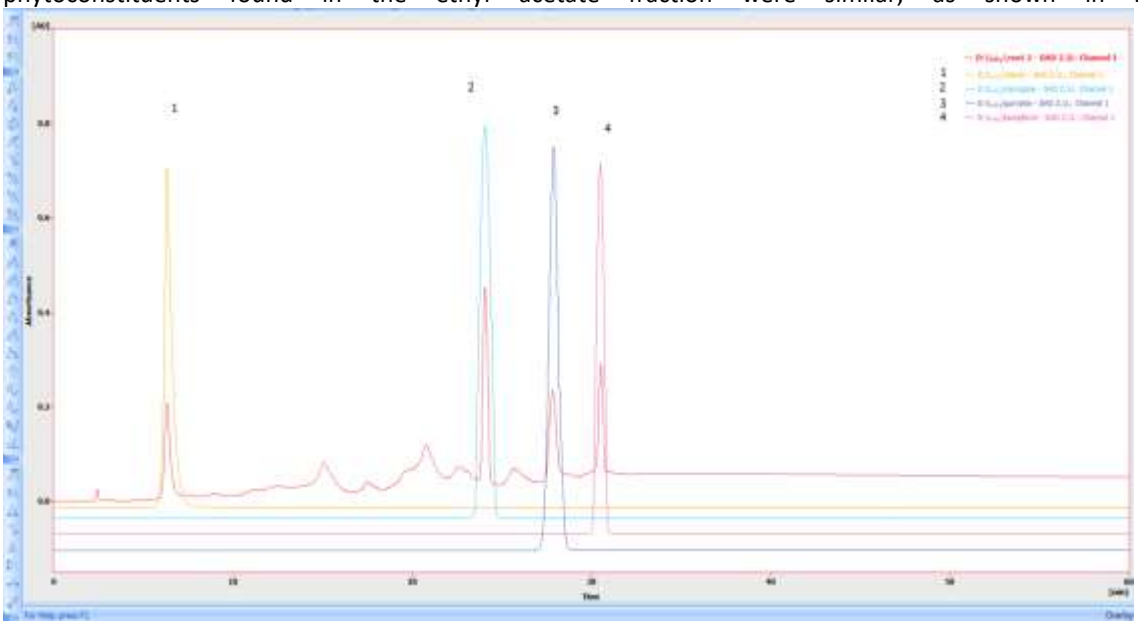


Figure (3): HPLC chromatogram of Euonymus japonicus roots (ethyl- acetate fraction).

An optimal correlation was observed between the UV spectra of ethyl acetate and four phytochemical standards: Naringenin, Vitexin, Kaempferol, and Quercetin, as illustrated in Figure 4. This suggests the presence of these phytochemicals in the Iraqi plant Euonymus japonicus.(18)

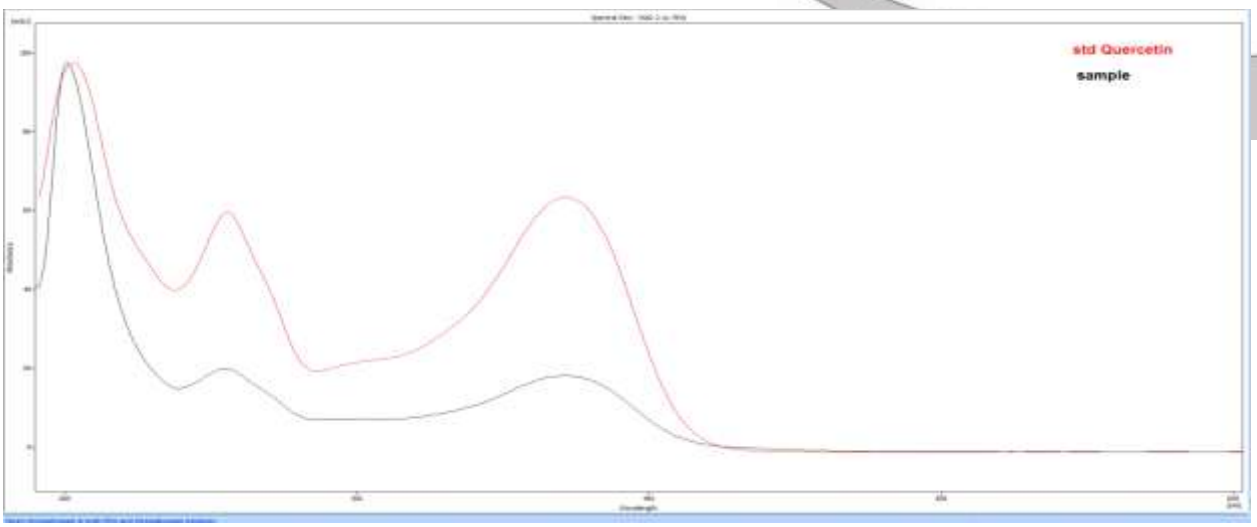


Figure (4): UV spectrum matching fitness of ethyl acetate fraction with quercetin standards.

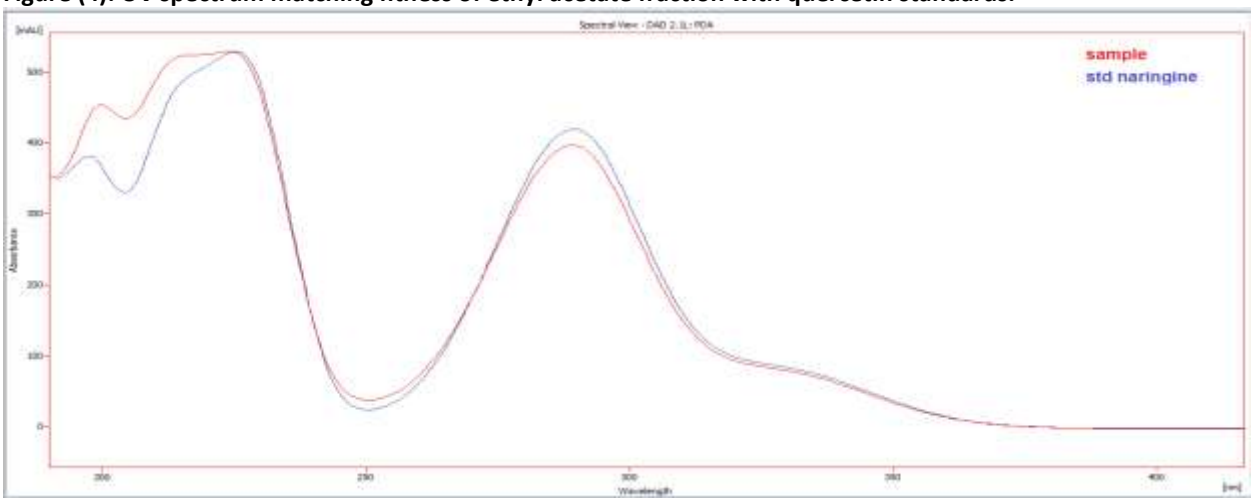


Figure (5): UV spectrum matching fitness of ethyl acetate fraction with Naringenin standards.

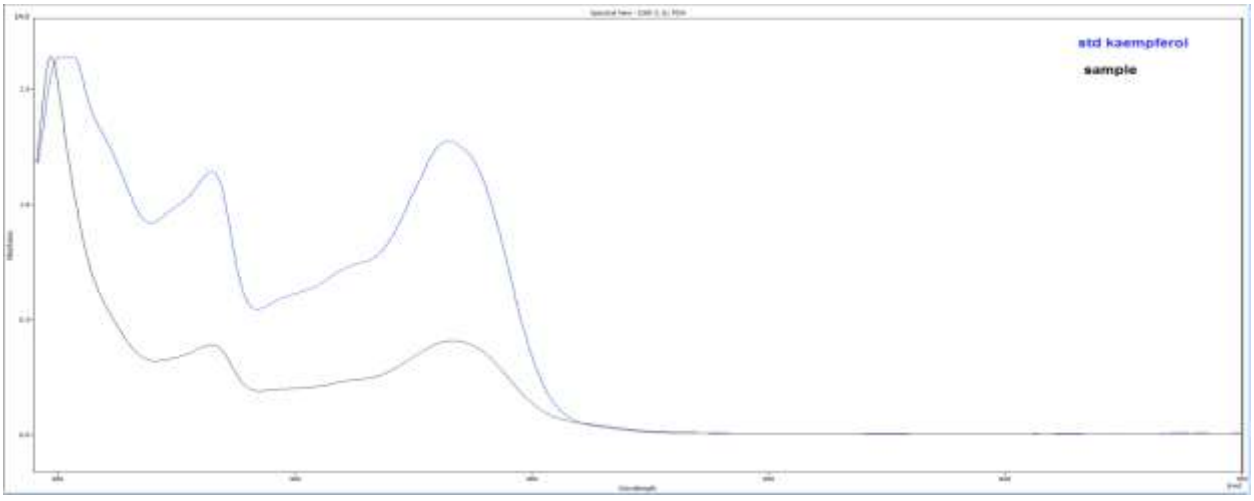


Figure (4): UV spectrum matching fitness of ethyl acetate fraction with Kaempferol standards.

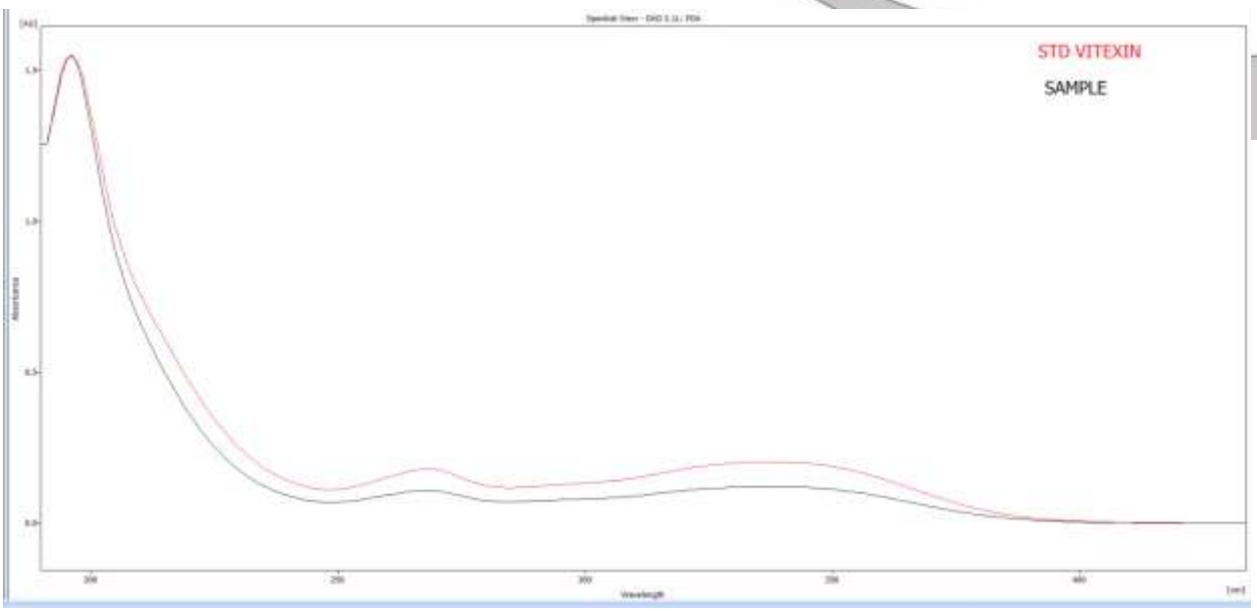


Figure (4): UV spectrum matching fitness of ethyl acetate fraction with vitexin standards.

It is important to examine the chromatogram of the ethyl acetate fraction, as it reveals several significant chemicals that did not meet the established criteria, highlighting the need for more thorough investigations with additional benchmarks. In the HPLC method, the area under each peak corresponds to the concentration of specific compounds. Therefore, calibration curves for five standardized substances were utilized for quantitative analysis, and peak areas from the ethyl acetate fraction were employed to determine their concentrations using the linear equation Y = aX + b, where slope (Y) represents the area under the curve and X denotes concentration in mg/mL. (19). Naringenin exhibited the highest quantitative levels among the five polyphenolic compounds present in the ethyl-acetate fraction, while quercetin displayed the lowest concentration, as shown in Table 2.

Table (2) The concentration of the proposed vitexin and Apigenin ,Naringenin , quercetin ,Kaempferol from roots of Ethyl acetate fraction of the plant

Compound in roots	Peak area	µg/ml	µg/mg extract
Vitexin	70.89	2.368356	0.110156093
Naringenin	94.415	4.5607975	0.212130116
Quercetin	30.705	0.7	0.037100814
Kaempferol	84.117	3.8076447	0.177099753

Four polyphenolic compounds—Naringenin, Vitexin, Quercetin, and Kaempferol—were identified in significant quantities. Vitexin is well-known for its properties, including antioxidant, anti-inflammatory, cytotoxic, antinociceptive, and neuroprotective effects (20). Quercetin plays a prominent role in various activities such as cytotoxicity, antioxidant action, anti-inflammation, anti-aging benefits, and neuroprotection (21), while Naringenin demonstrates anticancer properties (22). These diverse functions indicate that Euonymus japonicus holds promise for future pharmaceutical research due to its array of secondary metabolites, especially the polyphenolic constituents that were documented for the first time in this investigation.

Table(3) :Rf value of isolated component isolated from the roots of Euonymus japonicus .

Sample	**R_f value**
Kaempferol	0.70

Quercetin	0.76
Naringenin	0.78
Vitexin	0.25

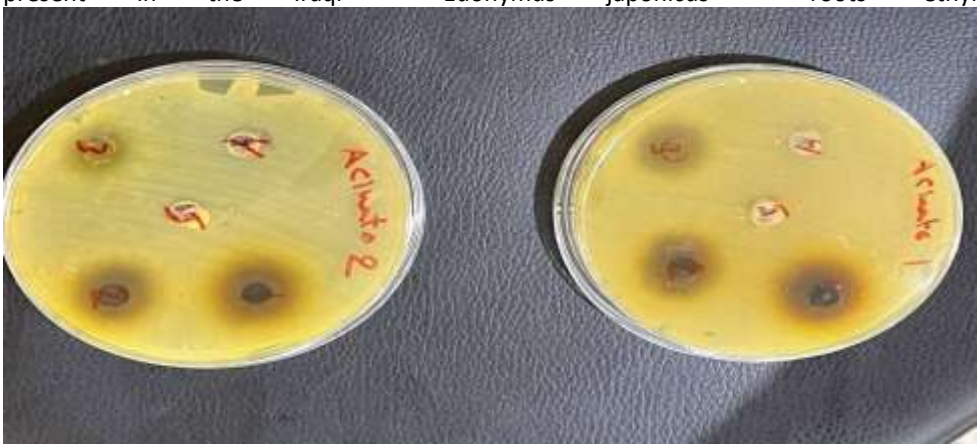
3. 3 Antibacterial activities of Euonymus japonicus L.

In recent years, the misuse and excessive use of antibiotics have led to an increase in resistance to available antibiotics. As a result, it has become crucial to explore new and more effective antimicrobial drugs that are also less harmful. This involves isolating and screening plants rich in active compounds for their therapeutic potential. (24)(25) .

The presence of inhibitory zones was leveraged to assess the antibacterial effectiveness of the ethyl acetate fraction derived from Euonymus japonicus roots against the microorganisms used in this investigation. (26). As illustrated in (Figure 6) and (Table 4). the ethyl acetate fraction at a concentration of 200 g/ml demonstrated a significant inhibitory effect against Streptococcus pneumoniae, with inhibition zones ranging from (20 to 23 mm). This effect was comparable to that of the positive control antibiotic, cefixime, which had an inhibition zone of (23 mm). Additionally, Actinomyces naeslundii exhibited notable activity with inhibition zones between (10 and 13 mm)at a concentration of (50 mg/ml), while cefixime showed no activity (0 mm).

Moreover, the antibacterial assessment indicated that the ethyl acetate fraction at 200 g/ml potentially affects gram-negative bacteria as well. It revealed a strong inhibitory effect against Morganella morganii , with inhibition zones measuring between (15 and 20 mm), whereas the positive control antibiotic (cefixime) recorded an inhibition zone of (0 mm).

In this investigation, the roots of Euonymus japonicus (specifically the ethyl acetate fraction) exhibited superior antibacterial properties against both gram-positive and gram-negative bacteria. These results may be linked to key components identified for the first time in this study, which include naringenin, vitexin, kaempferol, and quercetin present in the Iraqi Euonymus japonicus roots' ethyl acetate fraction.



A

B

C

Figure(6): antibacterial activity of ethyl acetate of root of Euonymus japonicus plant .A: for Actinomyces naeslundii ,B: Streptococcus pneumoniae, C :Morganella morganii

Table (4): the antibacterial activity of six concentration of ethyl acetate

Concentration of extract µg/ml	Average of inhibition zone diameter (mm)					
	Streptococcus pneumonia		Actinomyces naeslundii		Morganella morganii	
200	23	20	0	0	15	20
100	20	20	0	0	10	15
50	20	15	13	10	15	10
cefixime (positive control)	23	10	0	0	0	0

DMSO (Negative control)	**NA**

NA: no activity

CONCLUSION

In this study, the RP-HPLC method, utilizing a UV-vis facility alongside a range of polyphenolic standards, facilitated the identification and quantitative analysis of four flavonoid-class polyphenolic compounds in the ethyl acetate fraction of Iraqi Euonymus japonicus roots. Naringenin was found to be the most concentrated component, followed by Kaempferol, vitexin, and Quercetin, which exhibited lower concentrations. Furthermore, the plant demonstrated significant in vitro antibacterial activity, likely attributed primarily to the polyphenolic compounds identified through the agar well diffusion method.

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