

Research Article

To Study the Antioxidant and Antibacterial Activity of Leaf *R. communis* L. Extract against *Escherichia coli* and *Staphylococcus aureus* Isolated from Clinical Specimen

Zeena Yaqub Mahmood¹, Kamil M AL-Jobori²

^{1,2}Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq

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Corresponding Author:

Zeena Yaqub Mahmood, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Iraq

E-mail Id:

zema.Yaaqoub1100a@ige.uobaghdad.edu.iq

Orcid Id:

<https://orcid.org/0009-0007-6716-1609>

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A B S T R A C T

Introduction: One of the biggest challenges facing the world today is the rise of multidrug-resistant (MDR) bacteria. Two of the most common pathogens that develop resistance are *Escherichia coli* and *Staphylococcus aureus*. *Ricinus communis* is a medicinal plant that has been shown to have therapeutic benefits in treating a wide range of illnesses.

Aim: To assess the antioxidant and antibacterial activity of crude extract of the leaf of *R. communis* against two pathogenic bacteria (*E. coli* and *S. aureus*), isolated from clinical specimens.

Study Design: The antibacterial assay was conducted using a completely randomized design (CRD), with each treatment performed in triplicate. The crude leaf extract was tested at concentrations ranging from 0 to 400 mg/mL against the tested bacterial isolates. The 0 mg/mL treatment served as the negative control, ethanol was used as the solvent control where applicable, and standard antibiotics were used as positive controls.

Methodology: A total of 140 clinical specimens were collected from patients hospitalized in Medical City in Baghdad during October 2023 to January 2024. The specimens were cultured on blood agar, chocolate agar, Mannitol agar and MacConkey agar. The antioxidant activity of the leaf extract was assessed using the DPPH free radical-scavenging assay. The crude leaf extract of *Ricinus communis* was prepared at concentrations ranging from 0 to 400 mg/mL and tested for antibacterial activity against the bacterial isolates. The bacterial suspensions were exposed to the different extract concentrations, while a standard antibiotic was included as a positive control. The minimum inhibitory concentration (MIC), the sub-minimum inhibitory concentration (Sub-MIC) and the minimum bactericidal concentration (MBC) of the extract were also tested against the same bacterial strains using broth micro dilution method.

Results: Out of the 140 clinical specimens, 48 (34.29%) grew *E. coli* and 45 (32.14%) yielded *S. aureus*. The bacteria were conventional bacteriological methods and the VITEK-2 system. In the bacterial isolates, identified by conventional tests and Vitek-2 system, 16S rRNA gene was detected by amplification using simple PCR. DPPH scavenging assay showed that the extract had a higher antiradical capacity ($IC_{50} = 70 \mu\text{g/ml}$) compared with vitamin C ($IC_{50} = 180 \mu\text{g/ml}$). All the tested bacterial isolates were susceptible to the leaf extract; *E. coli* was susceptible to the concentrations 12.5 to 300 mg/ml, while *S. aureus* was susceptible to the concentrations 100 to 300 mg/ml. The MIC values ranged from 6.25 to 50 mg/mL, Sub-MIC values ranged from 3.13 to 25 mg/mL, and MBC values ranged from 12.5 to 100 mg/mL for both *E. coli* and *S. aureus*.

Conclusion: The findings demonstrate that *R. communis* leaf extract possesses both antioxidant and antibacterial activities against *E. coli* and *S. aureus*. These results suggest that *R. communis* may represent a promising source of bioactive compounds with potential antibacterial and antioxidant applications.

Keywords: *R. communis*, *E. coli*, *S. aureus*, antioxidant activity, antibacterial activity

Introduction

The evaluation of the medicinal value of any plant depends on the biologically active constituents and their amounts (Shah and Abdul-Jalil, 2022,¹ The active components of plants have an important role in controlling and protecting against a wide range of human illnesses (Ali and Tawfiq, 2023,² Over time, humans learned how to use herbal materials to their benefit (Al-Baaj and Abdul-Jalil, 2022,¹). *Ricinus communis* is a medicinal plant that is found naturally. Its active components have demonstrated efficacy against a wide range of bacteria and fungi (Ghalem et al., 2020,³ Tannins, saponins, phenols, alkaloids, glycosides, phytates, fatty acids, steroids, oxalates, and volatile constituents are among the phytochemicals present in the extracts of different plants (Ekoja and Audu-Peter, 2023,⁴ *R. communis* has been used in traditional medicine for a variety of diseases (Kebede and Shibeshi, 2022,⁵ This medicinal plant and its derivatives have been used to treat wounds, fever, cholera, bronchitis, cough, cold, diarrhoea, and dysentery (Desouky et al., 2022,⁶ Some of the alkaloids, steroids, flavonoids, saponins, and tannins are known to work as good antioxidants (Nanda et al., 2023,⁷ Flavonoids are a major subclass of phenolic compounds that share several common physicochemical properties with other phenolic compounds.

Mahmod et al., 2022,⁸ The *R. communis* extract, which has high phenolic and flavonoid contents, showed significant antioxidant activity in DPPH* scavenging (IC_{50} : 250.10) (Hussain et al., 2021,⁹ According to the literature review, it has historically been used to treat a number of inflammatory

disorders (Ekoja and Peter, 2023,⁵; Pansare and Deora, 2023,¹⁰ According to Jomova et al. (2023,¹¹ antioxidants are essential for regulating the amount of free radicals and other reactive molecules in the body and preventing oxidative damage. *R. communis* has anti-inflammatory properties and strong antioxidant components, which support its widespread use in traditional medicine (Panwar et al., 2023,¹²

In recent years, the emergence and dissemination of multidrug-resistant (MDR) *Escherichia coli* and *Staphylococcus aureus* have become significant global public health concerns. These pathogens are responsible for both community-acquired and healthcare-associated infections and can affect individuals across different age groups. The increasing prevalence of antimicrobial resistance has consequently reduced the effectiveness of conventional antimicrobial therapies and highlighted the urgent need to explore alternative antimicrobial agents (Meng et al., 2020,¹³; Assafi et al., 2022,¹⁴ An extensive range of diseases in both humans and animals are associated with the opportunistic bacterium *Staphylococcus* (Saeed et al., 2022,¹⁵ One of the most frequent causes of hospital acquired infections and epidemic community acquired infections (CA-MRSA) is *S. aureus*. It can cause necrotizing pneumonia, furunculosis, and necrotic lesions of the skin and soft tissues, among other severe clinical symptoms (Ahmad et al., 2020,¹⁶ The most effective antibacterial activity against *Candida albicans* and clinical isolates of *E. coli* and *S. aureus*, was demonstrated by the methanol crude extract of *R. communis* (Kebede and Shibeshi, 2022, 5). According to Mboyazi et al. (2020,¹⁷ the methanol extract of *R. communis* leaf exhibited antibacterial activity against *E. coli* bacterial strains, with MIC values of 6.25 mg ml⁻¹.

Iraq is among the nations that use antimicrobial agents widely. It has been hypothesised that excessive use of antibiotics is making microorganisms resistant to antibiotics. These resistant microorganisms can be transmitted and affect people through the food chain. It was this concern which drove this study.

Materials and Methods

This research is an experimental study that uses a completely randomized design (CRD) with three replications. A total of 140 clinical specimens (80 blood and 60 urine specimens) were collected from patients hospitalized at Medical City in Baghdad, over a four-month period from October 2023 to January 2024. The results of the study were analyzed using ANOVA with a significance level of 0.05.

Isolation and Diagnosis of *E. coli* and *S. aureus* bacteria

The samples were inoculated on blood agar, chocolate agar and MacConkey agar and the petri dishes were incubated at 37°C for 24-48 hours. The isolates were

identified based on colony characteristics or morphology after which pure colonies were sub-cultured on blood agar, Mannitol salt agar, MacConkey agar and chocolate agar. Further identification or confirmation of isolates was done by standard bacteriological techniques as stated by Usanga et al.(2020,¹⁸ Presumptive positive *E. coli* and *S. aureus* isolates were then confirmed using the VITEK-2 System (bioMerieux, Marcy l'Etoile, France) according to manufacturer's directions.

Molecular Identification

DNA was extracted using a DNA extraction mini kit (Intron, Korea) according to the manufacturer's protocol. The quality and quantity of extracted DNA were determined using a NanoDrop spectrophotometer (Dihan, Korea). The extracted DNA was stored at -20°C and used for molecular identification. The 16S rRNA gene was targeted by PCR as a complementary molecular approach to conventional bacteriological identification and VITEK-2 identification. PCR products were evaluated by agarose gel electrophoresis; no sequencing procedure was performed.

PCR reaction volume used for the detection of *E. coli* and *S. aureus* was set to be 25 µl. It contained 4 µl (100 ng) of genomic DNA, 1 µl (10 pmol) of each forward and reverse primer, 12.5 µl of Master Mix and 6.5 µl of nuclease-free water. PCR cycling conditions were as follows: 95°C for initial denaturation for 5 minutes, followed by 35 cycles of 1 minute at 94°C for denaturation, 45 seconds at 54°C and 55°C for annealing of *S. aureus* and *E. coli*, respectively, and 2 minutes at 72°C for extension, with a final extension at 72°C for 4 minutes. After PCR, products were separated by agarose gel electrophoresis, visualized by UV transillumination, and recorded using a digital camera.

Preparation of extract from leaves of *R. communis*

Leaves were washed thoroughly with distilled water to remove dust, and dried in a shady place at room temperature. The dried plant material was ground to fine powder using an electric grinder and stored at room temperature in the air tight containers in the dark for further use. 75 grams powder of leaves was mixed with 600ml of 70% methanol as solvent in the Soxhlet apparatus for 8 hours. The resulting extract was filtered using Whatman filter paper and the solvent was evaporated under vacuum by rotary evaporator

at 60°C for 4 hours. The extract in semi-solid state was collected in an air tight container and stored at 40 °C till further analysis (Alom and Das, 2018, 19)

DPPH Assay

The antioxidant activity of the prepared *R. communis* leaves extracts was evaluated conducted as follows: 0.04 g of DPPH was dissolved in 100 ml of methanol (400 µg ml⁻¹). To prepare the standard solution (vitamin C), 0.5 g of vitamin C was taken and mixed with 100 ml of methanol and distilled water. The concentration of the standard solution was 5000 ppm and using the dilution law, the other concentrations were prepared (30, 60, 120, 250, 500 ppm) from vitamin C and (30, 60, 120, 250, 500 ppm) from *R. communis* extract. About 1250µl of freshly prepared methanolic solution of DPPH was added to 50µl of each test solution, and the mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm by using spectrophotometer (Ahmed, 2013, 20). The IC₅₀ value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity (Koleva et al., 2002, 21).

The percent DPPH scavenging effect was calculated by using the following equation:

$$\text{DPPH scavenging effect (\%)} \text{ or percent inhibition} = \frac{A_0 - A_1}{A_0} \times 100.$$

Where: A₀ is the absorbance of blank, and A₁ is the absorbance in presence of test sample.

Antibacterial Activity Test

The antibacterial activity of *R. communis* extract against the isolated pathogens was tested by mixing the bacteria with the extract. Petri dishes were inoculated with each isolate. The petri dishes were then incubated at 37°C for 24 hours. The petri dish was divided from the center into four parts. The bacterial colonies in one of the parts were counted and the number was multiplied by four to obtain the total number of colonies in the petri dish. Antimicrobial activity was determined using the following equation: Number of bacterial colonies (%) = (Number of bacterial colonies in treatment / Number of bacterial colonies in control) x 100

$$\text{Inhibition (\%)} = 100 - \text{Number of bacterial colonies (\%)}$$

Table I. Primers Used for 16S rRNA Gene Amplification and Molecular Identification of *E. coli* and *S. aureus*

Bacterial species	Target gene	Primer	Sequence (5'–3')	Amplicon size (bp)	Reference
<i>E. coli</i>	16S rRNA	Forward (F)	5'AGAGTTTGATCMTGGCTCAG3'	919bp	Safarpour et al.,2020) (47)
		Reverse (R)	5'CCGTCAATTCATTTGAGTTT3'		
<i>S. aureus</i>	16S rRNA	Forward (F)	5'AACCTACCTATAAGACTGGG3'	570bp	(Musa,2024) (48)
		Reverse (R)	5'CATTTCACCGCTACACATGG3'		

For each bacterial isolate, controls were maintained in which dimethyl sulfoxide was used as a negative control and the discs of streptomycin (25 µg/disc-1), ampicillin (25 µg/disc-1) and gentamicin (10 µg/disc-1) were used as a positive control. The experiment was done three times.

Determination of MIC and MBC of *R. communis* extracts

To determine the antibacterial activity of *R. communis* extract against *E. coli* and *S. aureus*, a broth microdilution assay was employed to determine the minimum inhibitory concentration (MIC), sub-minimum inhibitory concentration (Sub-MIC), and minimum bactericidal concentration (MBC). Sterile 96-well flat-bottom polystyrene microplates were used. A 400 mg/mL stock solution of the *R. communis* extract was prepared in ethanol and subjected to two-fold serial dilution with brain heart infusion broth (BHIB) containing the bacterial inoculum. The final extract concentrations used for the assay were expressed in mg/mL. Appropriate controls were included on each microplate. Ethanol without bacterial inoculum was used as the solvent control, while sterile BHIB with the appropriate assay reagent and without bacterial inoculum served as the negative/growth control.

A well containing the plant extract and assay reagent without bacterial inoculum was included to control for any color interference caused by the extract. Plates were incubated at 37°C for 24 hours. Resazurin solution (30 µl) was then added to each well and incubated at 37°C for 30 minutes. Blue/purple color indicated inhibition of bacterial metabolic activity, whereas pink/colorless color indicated bacterial growth. The MIC was recorded as the lowest extract concentration showing no detectable growth by the assay. The Sub-MIC was defined as the concentration immediately below the MIC. The MBC was determined as the lowest concentration showing no viable bacterial growth according to the MBC assessment.

Results and Discussion

Isolation and Identification of *E. coli* and *S. aureus*

Out of the 140 specimens, 80 were blood specimens and 60, urine specimens. These were analyzed using standard bacteriological techniques. *E. coli* isolates were the most frequent at 48 (34.29%), followed by *S. aureus* at 45 (32.14%) (Table 2).

Table 2. The occurrence of *E. coli* and *S. aureus* in the different specimen types

Type of Specimens	No of specimens	E. coli			S. aureus		
		MacConkey agar	Blood agar	Chocolate agar	Mannitol agar	Blood agar	Chocolate agar
Blood	80	12	12	12	33	33	33
Urine	60	36	36	36	12	12	12
Total	140	48(34.29%)	48(34.29%)	48(34.29%)	45(32.14%)	45(32.14%)	45(32.14%)



Figure 1. *E. coli* on (A) MacConkey agar (B) Blood agar (C) Chocolate agar

It was observed that the *E. coli* colonies exhibited a pink to dark pink color on MacConkey agar as a result of lactose fermentation. The bacilli were long and thin, with a homogeneous stain (Figure 1A). *E. coli* displayed beta hemolysis and developed into grayish-white colonies on both Blood (Figure 1B) and Chocolate (Figure 1C) agar. The colonies from this media had comparable morphologies

in the gram stain; the bacilli were consistently marked thin and short.

Mannitol salt agar is a medium for differential growth and selective enrichment. When *S. aureus* was first cultured on Mannitol Salt Agar (MSA) and incubated for 24 hours at 37°C, it generated round, yellow, and pinkish colonies (Figure 2A). On blood agar, *S. aureus* produced round,

smooth, white to yellowish colonies, with hemolytic activity observed around some colonies (Figure 2B). On chocolate agar, *S. aureus* produced smooth, creamy to milky-white colonies (Figure 2C). These cultural characteristics were considered together with the results of the identification tests for confirmation of *S. aureus*.

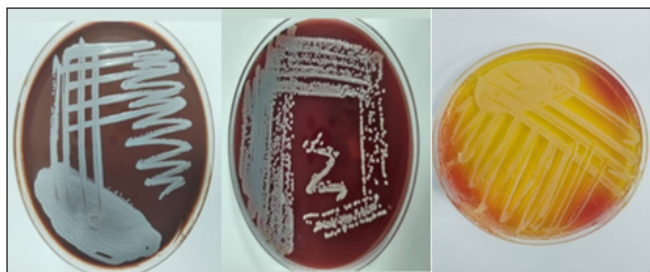


Figure 2. *Staphylococcus aureus* on (A) Mannitol Salt Agar (B) Blood Agar (C) Chocolate Agar

Using each primer pair to identify the particular target gene, single-target PCR reactions were carried out in order to maximize the amplification of the DNA templates for the PCR assay. Each of the primer pairs amplified single-target genes, encompassing the 16S rRNA present in each bacterium. According to the gel electrophoresis results, the size of the amplicons was 578 bp (16S rRNA gene) for all 48 *E. coli* isolates and 919 bp (16S rRNA gene) for all 45 *S. aureus* isolates (Figures 3;4). These results validated the Vitek-2 system tests and bacteriological methods.

PCR amplification of *E. coli* and *S. aureus* genes. PCR products were separated on a 1.5% agarose gel stained with ethidium bromide (EtBr). M: 100-bp DNA ladder marker. Lanes 1–10 show the 578-bp PCR products of *E. coli*, while lanes 1–10 show the 919-bp PCR products of *S. aureus*

Results showed that the extract exhibited a dose-dependent activity in scavenging DPPH radicals. Notably, the extract had a radical scavenging activity which was higher ($P \leq 0.05$)

than vitamin C at all concentrations. There was a gradual increase in scavenging activity with the increase in concentration of leaf extract. The extract demonstrated concentration-dependent DPPH radical scavenging activity from 30 to 500 $\mu\text{g ml}^{-1}$. The inhibitory percentages were 30.25 at 30 $\mu\text{g ml}^{-1}$ to 95.44 at 500 $\mu\text{g ml}^{-1}$ (Table 3). The results show that the leaf extract of *R. communis*, with an IC_{50} value of 70 $\mu\text{g ml}^{-1}$, possesses stronger anti-oxidant activity as compared to vitamin C (180 $\mu\text{g ml}^{-1}$).

Antibacterial activity

The antibacterial activity was evaluated by determining the inhibition percentage for the extract of *R. communis*, which showed significant antimicrobial activity against *E. coli* and *S. aureus*. Clear inhibition was observed for the leaf extract of *R. communis* as demonstrated in Figures 5 and 6. The zone of inhibition was clearly seen using extract at the concentrations of 12.5 to 300 mg ml^{-1} with the rate of inhibition 96-97, 97-98, 98-99, 94-95, 90-91 and 85-86%, respectively for *E. coli*, and at 100 to 300 mg ml^{-1} with the rate of inhibition 91-93, 84-86 and 84-86%, respectively for *S. aureus* (Table 4). The concentration of 400 mg ml^{-1} did not show activity (0%) against *E. coli* and *S. aureus* isolates.

MIC, Sub-MIC and MBC

The isolates, whether *E. coli* or *S. aureus*, differed in their response to the extract. The minimum bactericidal concentration (MBC) was determined as the lowest concentration at which no viable bacterial growth was detected following the MBC assessment. The MBC of the extract ranged from 12.5 to 100 mg mL^{-1} for both *E. coli* and *S. aureus*. The minimum inhibitory concentration (MIC) of the extract ranged from 6.25 to 50 mg mL^{-1} , while the sub-minimum inhibitory concentration (Sub-MIC) ranged from 3.13 to 25 mg mL^{-1} (Table 5).

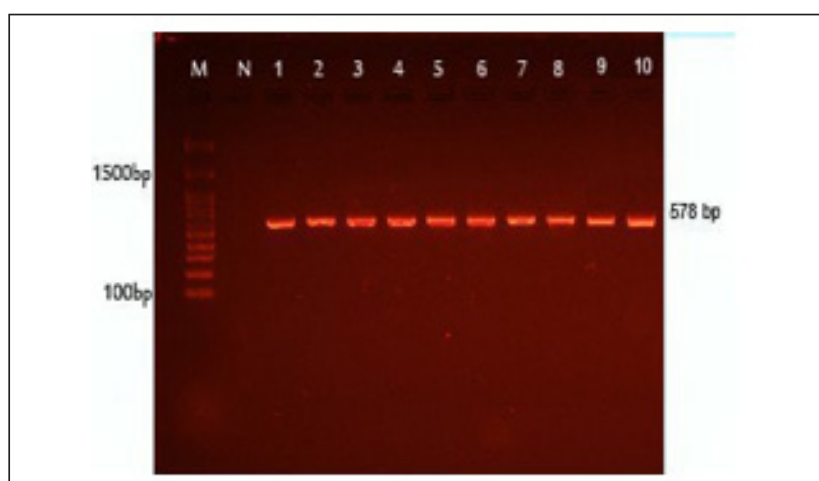


Figure 3. The amplification of 16S rRNA

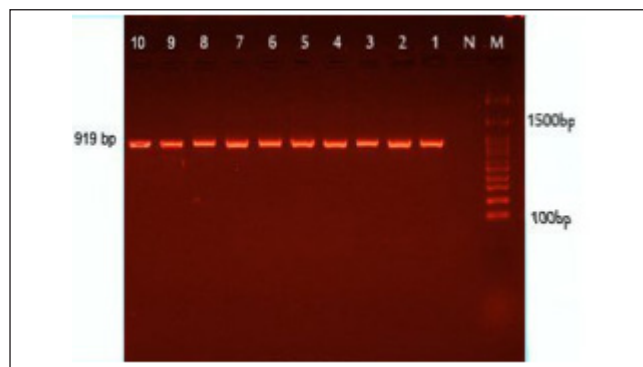


Figure 4.The amplification of 16SrRNA

Table 3.Radical scavenging activity of leaf *R. communis* extract

Conc. Treat.	30 ppm	60 ppm	120 ppm	250 ppm	500 ppm	LSD
Vit C	19.58	33.65	48.98	70.11	83.65	12.274*
Extract	30.25	49.88	78.98	87.08	95.44	14.983*
LSD	5.94*	7.03*	7.58*	8.51*	7.19*	-----
	P(≤0.05).*					

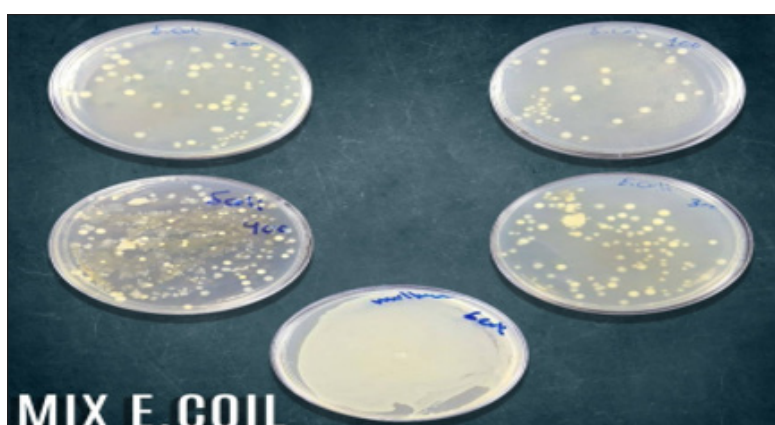


Figure 5.Antibacterial activity of leaf extracts against *E.coli*

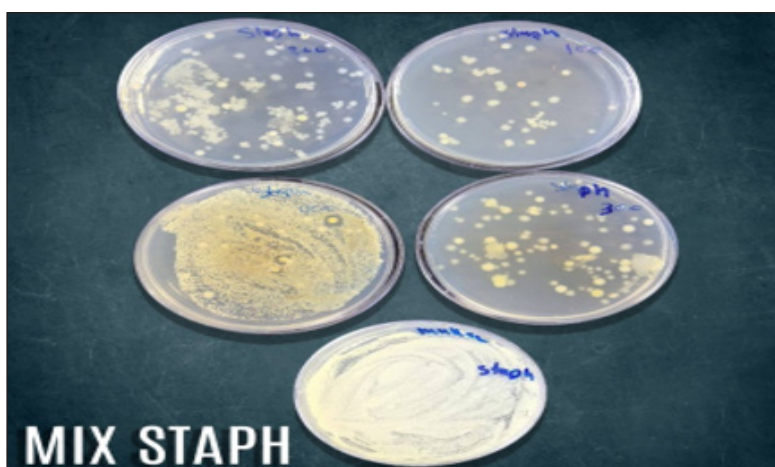


Figure 6.Antibacterial activity of leaf extracts against *S. aureus*

Table 4. Rate of inhibition percentage (%) of *R. communis* leaf crude extract against *E. coli* and *S. aureus*.

Number and type of bacterial isolates	Extract concentration and rate of Inhibition percentage (%)								LSD 0.05
	0	12.5	25	50	100	200	300	400	
<i>E. coli</i> (20)	0	96-97	97-98	98-99	94-95	90-91	85-86	0	7.68-8.14 *
<i>S. aureus</i>	0	0	0	0	91-93	84-86	84-86	0	9.76-11.78 *
* (P≤0.05) NS= Not Significant,									

Table 5. MBC, MIC and Sub-MIC of leaf *R. communis* extract on *E. coli* and *S. aureus* isolates

Isolate	<i>E. coli</i>			<i>S. aureus</i>		
	MBC	MIC	Sub-MIC	MBC	MIC	Sub-MIC
1	25	12.5	6.25	100	50	25
2	12.5	6.25	3.13	25	12.5	6.25
3	50	25	12.5	50	25	12.5
4	50	25	12.5	50	25	12.5
5	50	25	12.5	50	25	12.5
6	25	12.5	6.25	50	25	12.5
7	100	50	25	12.5	6.25	3.13
8	100	50	25	25	12.5	6.25

Discussion

Vitek-2, 16S rRNA gene PCR amplification, and traditional culture-based methods were used to identify the bacterial isolates.

Natural antioxidants that are necessary for survival against reactive oxygen species can be found in traditional medicinal plants. The extract demonstrated strong antioxidant activity against molecules of DPPH free radicals in polar solvent systems. Results of the present study indicate a gradual increase in scavenging activity with the increase in extract concentration (Table2). This is supported by a previous study by Hussain et al. (2021).⁹ which also found a gradual increase in scavenging activity with increasing extract concentration. The presence of secondary chemicals in *R. communis* leaves may be the cause of their antioxidant effect. Some bioactive compounds such as alkaloids, steroids, flavonoids, saponins and tannins are known to act as effective antioxidants (Nanda et al., 2023).⁷

Antioxidant chemicals generated by traditional medicinal plants play a crucial function in preventing critical molecules from being destroyed in a healthy system by neutralizing free radical molecules and stopping the chain reaction (Chaudhary et al., 2023).²² Antioxidants are found in considerable quantities in the leaves of *R. communis*. On the other hand, by removing free radicals from the biological system through their hydroxyl moieties on the

phenolic ring, phenolics have been demonstrated to protect disorders linked to oxidative stress (Kruk et al., 2022).²³ Also, leaf saponins and root phenolics are potential antioxidative stress compounds as they protect the RBCs from hemolysis with an efficacy which is close to butylated hydroxyl anisole (BHA) (Inayor and Ibraheem, 2014).²⁴

A low IC₅₀ value suggests that the extract has a significant DPPH scavenger activity. *R. communis* appears to be a better radical scavenger with an IC₅₀ of 70 µgml⁻¹, in comparison to Vitamin C with an IC₅₀ value of (180) µgml⁻¹. According to Mounira (2015),²⁵ the EAE had the strongest radical scavenging activity with an IC₅₀ of 0.044± 0.001 mgml⁻¹, which was noticeably close to BHT's 0.032± 0.000 mgml⁻¹. Organic extract from *R. communis* was found to have a low IC₅₀ of 945 mgml⁻¹ against a stable free radical (DPPH) (Mongalo et al., 2018).²⁶ Comparing these results with the current study is difficult because in addition to variations in the extraction solvent employed, geographic location, and collecting periods, there is a significant variation in the quantity of DPPH used across these studies.

Finding new, effective medications is crucial since bacteria have developed multi-drug resistance to traditional antimicrobial medicines. In this context, the present investigation demonstrates the significant potential of *R. communis* extract by affecting the growth and multiplication of the two harmful bacteria - *E. coli* and *S. aureus*.

Differential inhibitions of these two bacteria were observed when treated with the *R. communis* extract. Remarkably, the *R. communis* leaf extract exhibited strong inhibitory activity against *E. coli* at concentrations ranging from 12.5 to 100 mg/mL, resulting in 90–99% inhibition of bacterial growth. In contrast, *S. aureus* showed a lower susceptibility to the extract, with concentrations of 100–300 mg/mL producing 84–93% inhibition of bacterial growth (Figures 5 and 6; Table 3).

It has been extensively documented that *R. communis* possesses potential antibacterial activity (Kebede and Shibeshi, 2022, 5; Voleti et al., 2022.²⁷ These studies were conducted using crude extracts. Its ability to inhibit both Gram-positive *S. aureus* and Gram-negative *E. coli* bacteria suggests that it may have inhibitory effect on many other bacteria. Perhaps more significantly, though, is its potential to inhibit enteric pathogenic bacteria, which could help reduce the threat of antibiotic-resistant bacterial strains. In this regard, the current results (Figures 5; 6 and Table 3) demonstrate that *R. communis* produces significant antibacterial activity against Gram –negative (*E. coli*) and less against Gram- positive bacteria (*S. aureus*), which is consistent with the study by Ghani et al. (2023).²⁸ that showed *R. communis* produced high antibacterial activity against Gram-negative (*E. coli*) and less against Gram-positive bacteria (*S. aureus*). The structural variations in the cell surfaces of Gram-positive and Gram-negative bacteria may be the reason for this. The antibacterial activity of plant extracts, including basil essential oil, may be attributed to their ability to increase oxidative stress within bacterial cells, resulting in damage to intracellular macromolecules and ultimately leading to cell death (Ahmed et al., 2023).²⁰ Morphological variations in the bacterial cell wall's structure may have an impact on the potency of the phytochemicals present in the *Ricinus* leaf extract. Gram-negative bacteria have a thin peptidoglycan layer (2–3 nm) in between cytoplasmic membrane and the outer membrane when compared to Gram-positive bacteria which are devoid of the outer membrane but contain a peptidoglycan layer of 30 nm thickness. This thicker wall may act as a barrier protecting the cell from the entry of phytochemicals into the cytoplasm. The bacterial strain may also regulate the bioavailability of the phytochemicals present in the *R. communis* leaf extract by modification or inactivation (Hajrah et al., 2018).²⁹ In general, phenolics can target many sites in bacteria through their powerful mechanisms of action that interfere with bacterial cell wall construction, DNA replication, or enzyme production. This increases the sensitivity of bacteria to these naturally occurring chemicals (Lobiuc et al., 2023).³⁰

Varying doses of the *Ricinus* leaf extract (i.e. 200 to 1.56 µg. ml⁻¹) were tested against *E. coli* and *S. aureus* using the broth dilution method, and MIC, Sub-MIC and MBC. The MIC of the extract revealed antibacterial activity ranging

from 6.25 - 50 µg.ml⁻¹ against the tested bacterial strains. Different concentrations of *Ricinus communis* leaf extract, ranging from 200 to 1.56 µg/mL, were tested against *E. coli* and *S. aureus* using the broth dilution method. The minimum inhibitory concentration (MIC), sub-minimum inhibitory concentration (Sub-MIC), and minimum bactericidal concentration (MBC) were determined. The MIC values of the extract ranged from 6.25 to 50 µg/mL, indicating antibacterial activity against the tested bacterial isolates. The corresponding Sub-MIC values ranged from 3.13 to 25 µg/mL. Complete inhibition of bacterial growth, as indicated by the MBC, was observed at concentrations ranging from 12.5 to 100 µg/mL (Table 4). The MIC values of *R. communis* leaf extract against the pathogenic bacteria isolated from wound infections ranged up to 220 mg/mL, while the lowest MBC recorded was 300 mg/m (Voleti et al., 2022, 27). The MIC and MBC values of *R. communis* leaf extract on the isolated pathogens of the current study differed from the results of studies conducted in other countries (Suurbaar et al., 2017).³¹ Also, crude extracts of *R. communis* leaves showed significant antibacterial activity against tested microorganisms using quantitative microbroth dilution method with a range from 0.13 to 1.05 mgml⁻¹ (Nemudzivhadi, 2015).³² MIC depends on the microbe and the extract. Greater sensitivity was observed with methanol, ethanol and aqueous extracts while petroleum ether and ethyl acetate extracts were least sensitive with MBCs remaining undetected. In addition, antimicrobial properties also vary in different species of medicinal plants. Altitude, temperature, light, and moisture are other variables. Medicinal plants' secondary metabolite accumulation and metabolism are influenced by these parameters. The presence of various active substances and their concentrations are influenced by variations in the locations of medicinal plants (Liu et al., 2016).³³

Conclusion

16S rRNA gene-targeted PCR primer sets provided molecular support for the identification of *E. coli* and *S. aureus* in combination with conventional bacteriological methods and the VITEK-2 system.

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