



Extraction of flavonoids from *Saccharomyces cerevisiae* and *Sporobolomyces roseus* and evaluation of their antibacterial activity

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ABSTRACT

The aim of this study was to extract flavonoids from *Saccharomyces cerevisiae* and *Sporobolomyces roseus* and evaluate their antibacterial activity against selected bacterial pathogens. Flavonoids (F) were extracted from yeasts (*Saccharomyces cerevisiae* and *Sporobolomyces roseus*) using 70% acetone, followed by separation and purification through thin-layer chromatography (TLC) and column chromatography. The compounds were then identified using UV absorption spectroscopy and Fourier transform infrared (FTIR) spectroscopy. The result showed the final purified of flavonoids recorded 10% of flavonoid (F1) from *Saccharomyces cerevisiae* and 8% of flavonoid (F2) from *Sporobolomyces roseus*. UV absorbance spectroscopy and FTIR results showed that both F1 and F2 agents have polar natures with similar purified flavonoids basic backbone structures. F1 and F2 agents showed high antibacterial activities against all the tested bacteria (*Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus*). Agent F1 exhibited the lowest MIC range (7.8–31.25 µg/ml), while F2 had a consistent MIC of 62.5 µg/ml against all tested bacteria. These findings suggest that yeast-derived flavonoids have potential as alternative antimicrobial agents for combating antibiotic-resistant bacteria.

1. Introduction

Flavonoids are a diverse class of secondary metabolites known for their antimicrobial, antioxidant, and anti-inflammatory properties [1]. These polyphenolic compounds play a crucial role in plant defense mechanisms and have been widely studied for their potential therapeutic applications [2]. While plants have historically been the primary source of flavonoids, recent research has highlighted the ability of microorganisms, including yeast, to synthesize these bioactive compounds through various biosynthetic pathways [3,4].

Among microorganisms, yeasts have gained attention as potential flavonoid producers due to their fast growth, ease of genetic manipulation, and ability to produce secondary metabolites. *Saccharomyces cerevisiae*, a well-characterized yeast, has been widely used in synthetic biology to engineer flavonoid biosynthesis pathways [4]. Similarly, *Sporobolomyces roseus*, a lesser-known yeast species, has demonstrated the ability to synthesize various bioactive compounds, including carotenoids and flavonoids [3]. These yeasts present an untapped reservoir of bioactive metabolites that may hold promise for pharmaceutical and industrial applications.

One of the most pressing global health challenges today is antimicrobial resistance (AMR), which threatens the effectiveness of existing antibiotics [5]. The widespread misuse and overuse of antibiotics have led to the emergence of multidrug-resistant bacterial strains, making it increasingly difficult to treat common infections [6,7]. This has intensified the search for alternative antimicrobial agents, including natural products such as flavonoids, which have shown broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria [8].

The antibacterial mechanisms of flavonoids vary depending on their structure but generally include disrupting bacterial cell membranes, inhibiting essential enzymes, interfering with quorum sensing, and blocking nucleic acid synthesis [9,10]. While flavonoids from plants have been extensively studied for their antimicrobial properties, yeast-derived flavonoids remain largely unexplored.

Fungi produce flavonoids with different biological activities, such as *Sanghuangporus baumii* synthesized 81 flavonoids on a chemically defined medium [11]. Among 21 endophytic fungi were isolated for the first time from wild *Conyza blinii* H. Lév, There were six strains that produced flavonoids [12]. The flavonoids kaempferol, resokaempferol, quercetin, fisetin, liquiritigenin, and naringenin were identified in the yeast *Saccharomyces cerevisiae* [13].

Flavones and flavonoids were detected in *Penicillium chrysogenum* [14]. Investigating the antibacterial potential of flavonoids extracted from *S. cerevisiae* and *S. roseus* could provide new insights into their mechanisms of action and potential applications in combating drug-resistant bacteria.

This study aimed to extract and characterize flavonoids from *S. cerevisiae* and *S. roseus* and evaluate their antibacterial activity against selected bacterial pathogens. By exploring yeast-derived flavonoids as an alternative antimicrobial source, this research contributes to the growing body of knowledge on microbial biosynthesis of bioactive compounds and their potential role in developing novel antimicrobial agents. Given the urgent need for new antibacterial strategies, yeast-derived flavonoids could represent a promising natural solution to address the global threat of antibiotic resistance.

2. Materials and methods

2.1 Sampling and isolation of fungi

Five soil samples were collected from different locations in southern Basrah Province (Shaibah, AlTannumah, Al Khait, Banks of the Basrah canal, and Jabal Sanam). Fungi were isolated using the dilution plate method. One gram of each soil sample was mixed with 9 mL of sterile distilled water, serially diluted to 10^{-3} , and plated onto Potato Dextrose Agar (PDA) (Oxoid, England) supplemented with chloramphenicol. Plates were incubated at $28 \pm 2^\circ\text{C}$ for 10 days. Pure fungal isolates were sub-cultured and maintained on PDA slants at 4°C .

2.2 The phenotypic and molecular identification

Morphological identification was based on colony characteristics and microscopic features [15]. The identification of *Saccharomyces cerevisiae* and *Sporobolomyces roseus* was confirmed using CHROMagar (Himedia, India) [16] and further validated through molecular identification by amplifying the ITS1 and ITS2 regions using universal primers ITS1 and ITS4 [17]. Primer sequences and PCR conditions are detailed in Supplementary Tables 1–3. The DNA sequences of the PCR products were deposited in GenBank under accession numbers LC791407 (*S. cerevisiae*) and LC791408 (*S. roseus*).

2.3 Flavonoid extraction and purification

2.3.1 Primary screening of secondary metabolites from yeast.

Two yeast strains, *Saccharomyces cerevisiae* and *Sporobolomyces roseus*, were selected for flavonoid extraction. Six grams of yeast were harvested, carefully collected with a spatula from cultures incubated at $28 \pm 2^\circ\text{C}$ for 48 hours on Sabouraud Dextrose Agar (HiMedia, India). The harvested yeast was combined with 40 ml of 70% acetone and 150–300 mg of glass beads in a 100 ml conical flask. The flask was sealed with aluminum foil and placed on a stirrer for 48 hours to disrupt the cell walls. The mixture was then centrifuged to separate the cell debris from the extract [18].

2.3.2 Thin layer chromatography (TLC)

Silica gel-coated plates (20×20 cm, 2 mm thick) were used with a mobile phase of 1 part acetone and 2 parts benzene. Spots were visualized using iodine vapor. Rf values were calculated as $R_f = a/b$ [19].

a = The distance from the starting point to the gravity center of the sample spot.

b = The distance from the starting point to the front of the developing solvent [19].

2.3.3 Column chromatography

A glass column (50 × 6 cm) packed with 150 g silica gel (HiMedia, India) was used for separation. Flavonoids F1 (*S. cerevisiae*) and F2 (*S. roseus*) were dissolved in 1 ml distilled water and applied to the column. Elution used a mobile phase of 2 parts benzene and 1 part acetone at a flow rate of 1 ml/min. Fractions were collected and analyzed via TLC. Samples with similar Rf values were grouped together and collected into a single tube [20].

2.4 Identification of flavonoids

2.4.1 UV absorption spectroscopy

UV absorption spectroscopy was performed using a conventional quartz cell with a 1 cm optical path length at 298 K. Measurements for F1 and F2 were conducted with a PG T90U UV-Visible spectrophotometer on aqueous solutions prepared by dissolving 0.1 g of each sample in 10 ml of acetone [21].

2.4.2 Fourier transform infrared (FTIR) spectroscopy

The IR spectra of F1 and F2 were recorded using a Shimadzu IRAffinity-1 spectrometer with KBr discs, covering the range of 4000 to 500 cm^{-1} , following the method outlined by Silverstein et al. [21].

2.5. Identification of bacterial strains

The clinical isolates were obtained from Al-Sader Teaching Hospital which included *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*. The clinical isolates first identified based on their phenotypic characteristics using a series of biochemical tests. Their identification was then verified through polymerase chain reaction (PCR) using species specific primers. The detailed characterization of these isolates and the molecular identification methods applied, has been previously described in a published study [7]

2.6 Antimicrobial activity assays

The flavonoids (F1 and F2) extracted from *Saccharomyces cerevisiae* and *Sporobolomyces roseus* were tested for antimicrobial activity against some G+ and G- bacteria which included 119 Gram negative bacteria (53 *E. coli*, 25 *Klebsiella pneumoniae*, 41 *Pseudomonas aeruginosa*) and 48 Gram-positive bacterial strains (*S. aureus*) using the agar diffusion and broth microdilution methods.

2.6.1 Antimicrobial susceptibility testing

The antimicrobial susceptibility of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* (with 53, 25, 41, and 48 isolates, respectively) was assessed using the disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines [22].

The antibiotics tested in this study included Cefoxitin (30 μg), Ceftriaxone (30 μg), Cefotaxime (30 μg), Ceftazidime (30 μg), Cefepime (10 μg), Amoxicillin/clavulanic acid (30 μg), Piperacillin/Sulbactam (20 μg), Piperacillin/tazobactam (110 μg), Aztreonam (30 μg), Imipenem (10 μg), Meropenem (10 μg), Colistin (10 μg), Gentamicin (10 μg), Amikacin (30 μg), Ciprofloxacin (10 μg), Erythromycin (15 μg), Clindamycin (2 μg), Linezolid (30 μg), Tetracycline (10 μg), Oxacillin (30 μg), Methicillin (5 μg), Rifampin (5 μg), and Vancomycin (30 μg).

To ensure accuracy, the results of antibiotic susceptibility testing and minimum inhibitory concentration (MIC) determination were further validated using the VITEK 2 system.

2.6.2 Agar well diffusion assay for purified flavonoids.

The estimation of the antibacterial activity of flavonoids (F1 and F2) was studied against *E. coli* (53 isolates), *P. aeruginosa* (41 isolates), *K. pneumonia* (25 isolates), and *S. aureus* (48 isolates) using the agar well diffusion method [23]. Hundred μl of the bacterial culture suspension ($\sim 10^6$ cells/ml) was spread on Muller Hinton agar (MHA) (Himedia, India). Wells (6 mm in diameter) were created and filled with 100 μl of either flavonoid F1, flavonoid F2, or gentamycin (as a resistance control), each at a concentration of 125 $\mu\text{g/ml}$ (Flavonoids were dissolved in normal saline to prepare the working concentration (125 $\mu\text{g/ml}$), and gentamycin, originally in liquid form, was similarly diluted with normal saline to a final concentration of 125 $\mu\text{g/ml}$). The plates were then incubated at 37 °C for 24–48 hours, and antibacterial activity was determined by measuring the diameter of the inhibition zones (mm) [24]. The mean inhibition zone was calculated for each bacterial species based on all isolates tested, providing an average measure of the antibacterial effect for each pathogenic bacteria.

2.6.3 MIC and MBC determination

The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of flavonoids F1 and F2 were determined against eight multidrug-resistant (MDR) bacterial isolates (*E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*) using the standard broth microdilution method in 96-well plates, following CLSI guidelines [22]. The isolates tested were selected for their high sensitivity to flavonoids and included strains U21 and B7 (*E. coli*), U5 and S11 (*K. pneumoniae*), S10 and S53 (*P. aeruginosa*), and U39 and S69 (*S. aureus*).

Flavonoids and gentamicin (as a resistance control) were tested in concentrations ranging from 0.97 to 250 $\mu\text{g/ml}$. Bacterial growth was monitored at 0 and 24 hours by measuring optical density (OD600). MIC was defined as the lowest concentration inhibiting visible growth, while MBC was the concentration showing no bacterial growth on nutrient agar after incubation, based on the dropping method [25]. All tests were conducted in triplicate for accuracy.

2.6.4 Growth kinetics assay

As F1 exhibited the lowest MIC and MBC values, it was selected to investigate the impact of flavonoids on the growth kinetics of multidrug-resistant (MDR) *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *S. aureus*. The isolates tested were the same as those used in the MIC and MBC determination experiments. The inhibitory effects on bacterial growth were evaluated by measuring optical density (OD₆₀₀) over time. Two isolates from each species were tested in 96-well microtiter plates, with F1 applied in two-fold dilutions ranging from 0.25× MIC to 4× MIC (1.9 to 125 µg/ml). Cultures were incubated at 37°C with shaking at 200 rpm, and OD₆₀₀ readings were taken at 0, 60, 120, 180, 300, and 360 minutes to monitor growth during the early to mid-log phase over 6 hours. A negative control was prepared without the addition of F1 to the inoculum, while gentamicin was used as a resistance control. All tests were conducted in triplicate for accuracy.

3 Result

3.1 Morphological and molecular identification of fungi

The identification of *Saccharomyces cerevisiae* and *Sporobolomyces roseus* was based on phenotypic characteristics on CHROMagar, *Saccharomyces cerevisiae* appeared as purple colonies, whereas *Sporobolomyces roseus* produced orange-red colonies, then confirmed through the amplification of the ITS1-5.8S-ITS2 rDNA region (Supplementary Fig 1). BLAST analysis of the ITS1-5.8S-ITS2 rDNA sequences, which have been deposited in the GenBank database under accession numbers LC791407 and LC791408, revealed 100% identity with *S. cerevisiae* and *S. roseus*, respectively.

3.2 Flavonoid extraction and purification

After separation and purification using column chromatography, the purified flavonoids from yeast showed weights of 0.5g (10%) for F1 from *Saccharomyces cerevisiae* and 0.8g (8%) for F2 from *Sporobolomyces roseus*. The Thin Layer Chromatography (TLC) analysis revealed 6 spots for F1 (with RF values of 0.05, 0.27, 0.34, 0.54, 0.82, and 0.96) and 5 spots for F2 (RF values of 0.04, 0.06, 0.53, 0.81, and 0.88). During the purification process, samples were collected from the column at a flow rate of 1 ml per minute. Each sample was analyzed by TLC and checked using iodine vapor. Samples with similar RF values were pooled into one tube. The final RF values recorded were 0.97 for F1 and 0.87 for F2.

3.3 Identification of flavonoids

3.3.1 UV absorption spectroscopy of F1 and F2

The UV absorption spectra result for F1 and F2 agents indicated strong absorption for F1 at wavelengths of 249, 262, 274, 320, and 361 nm (Fig.1 and Table 1), while F2 showed absorption at 250, 262, 272, and 360 nm (Fig.1 and Table 1). These absorption bands correspond to π - π^* transitions of the benzene ring. Lower absorption values for F1 were recorded at 477, 452, and 465 nm, while for F2, they were observed at 476, 476, 449, and 465 nm, respectively. This lower absorption is associated with n - π^* transitions caused by groups like O-H.

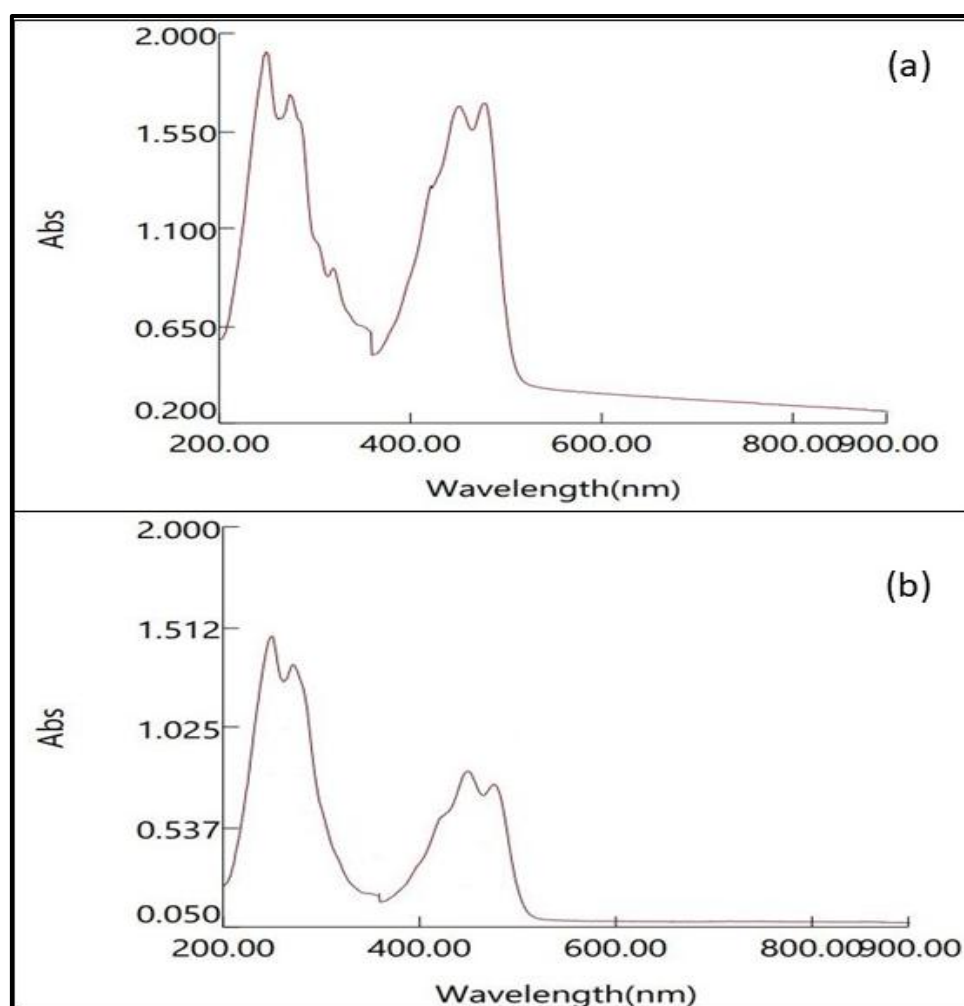


Fig.1. UV absorption spectrum of the F1 agent (a) for *Saccharomyces cerevisiae* and the F2 extract (b) for *Sporobolomyces roseus* (0.1 g/mL in acetone), measured using a UV-visible quartz cell with a 1 cm path length at 298 K. The results indicate that both the F1 and F2 agents exhibit polar natures and share similar basic flavonoid backbone structures

Table 1. UV absorption spectrum wavelengths (nm) for F1 and F2 agents.

Compound	π - π^* Wavelength (nm)	n - π^* Wavelength (nm)
F1	249,262,274,320,361	477,452,465
F2	250,262,272,360	476,449,465

π - π^* = For benzene ring, n - π^* = For ion pair of oxygen.

3.3.2 FTIR spectroscopy for F1 and F2.

FTIR spectroscopy was employed to characterize the structure of the F1 and F2 agents. The results of the FTIR spectra and their corresponding assignments are presented in Figure 2, as well as Tables 2 and 3, respectively.

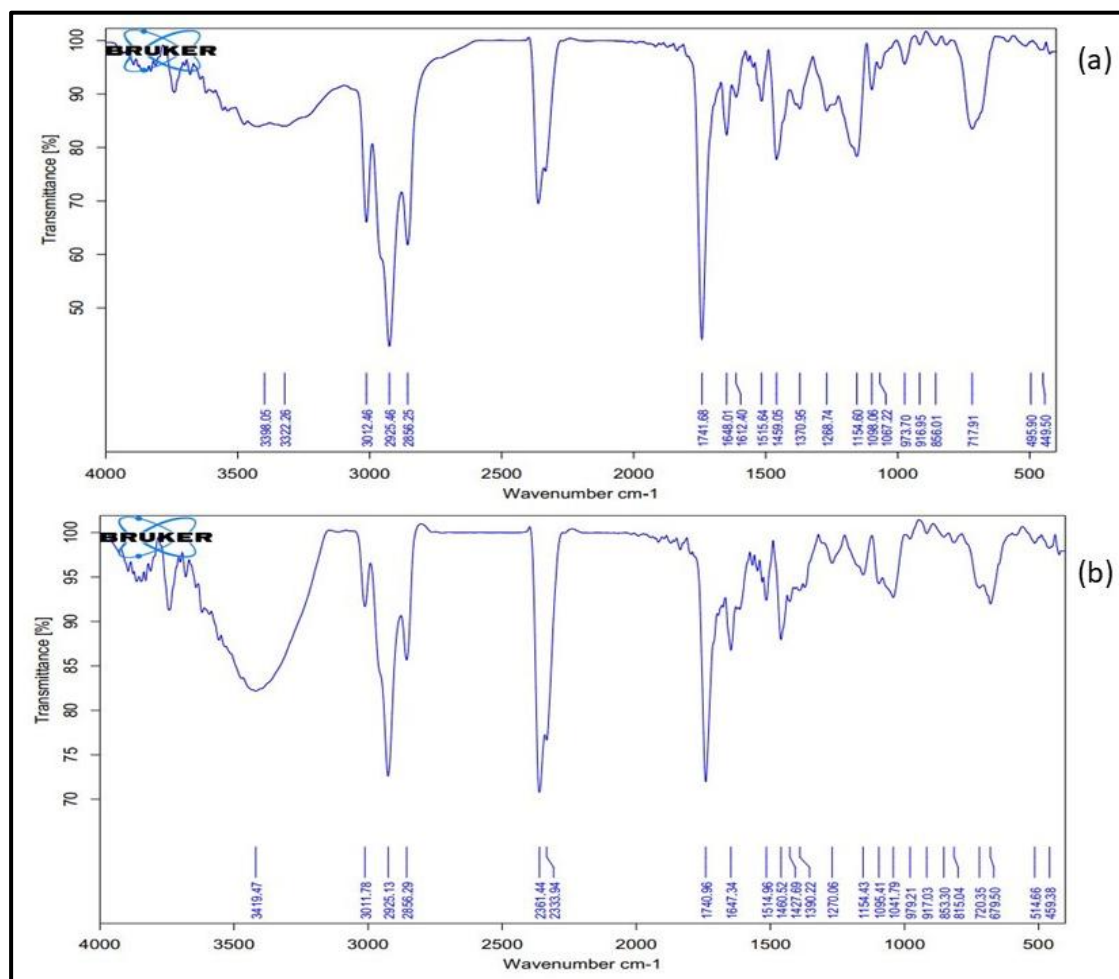


Fig. 2. Fourier transforms infrared results of F 1 (a) and F2 (b). The results showed that both F1 and F2 agents have polar natures with similar purified flavonoids basic backbone structures.

Table 2. The functional groups of purified F1 detected by FTIR

Wave number (cm-1)	Assignment
3396	O-H, strong
3012	Aromatic C-H, medium
3461	O=C=O, strong
1741	C=O, strong
1459	C=C, medium
1154	C=O, medium
717	Bending C=C, medium

Table 3. The functional groups of purified F2 detected by FTIR

Wave number (cm-1)	Assignment
3419	O-H, strong
3011	Aromatic C-H, weak
1740	C=O, strong
1460	C=C, medium
1270	C=O, weak
679	Bending C=C, weak

Both F1 and F2 exhibited nearly identical functional groups, suggesting that they may share similar structural characteristics (Fig. 3).

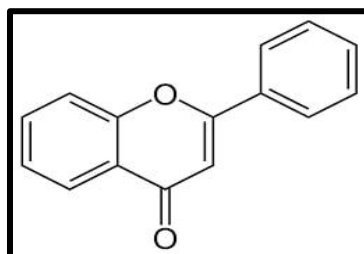


Fig. 3. Proposed backbone structures of F1 and F2 (polar flavonoids), based on the results Obtained from UV absorption spectra and FTIR analysis.

3.4 Antimicrobial activity assays

3.4.1 Bacterial identification and Antibiotic Resistance Patterns

All the tested isolates (53 *E. coli*, 25 *K. pneumoniae*, 41 *P. aeruginosa*, and 48 *S. aureus*), identified through routine microbial testing, were positive for species-specific PCR (Supplementary Fig 2). The antibiotic susceptibility results (Figures 4 & 5) revealed varying resistance patterns among the bacterial isolates. *E. coli* recorded the highest resistance to Cefoxitin (92.45%), Aztreonam (86.79%), and Piperacillin/Sulbactam (81.13%), while the lowest resistance was to Amikacin (3.77%), followed by Imipenem (15.09%). *P. aeruginosa* exhibited very high resistance to most β -lactams, with the highest against Piperacillin/Sulbactam (95.12%), Cefoxitin/Ceftriaxone (87.8%) and Cefotaxime (82.93%). Another noteworthy finding was carbapenem resistance (Imipenem 73.17%, Meropenem 70.73%). In particular, *K. pneumoniae* exhibited moderate to high resistance to Cefepime (64%), Amoxicillin/Clavulanic acid (64%), and Piperacillin/Sulbactam (68%), with lower resistance to Imipenem (28%) and Amikacin (20%). Overall, the isolates demonstrated multidrug resistance patterns, with amikacin and carbapenems remaining the most effective agents, while colistin was effective against *P. aeruginosa*.

In the case of *S. aureus* isolates, the highest resistance was observed against Cefoxitin (93.75%), (Figure 5). All Methicillin-resistant *S. aureus* (MRSA) strains tested were positive for the *mecA* gene.

A substantial proportion of multidrug-resistant (MDR) strains was detected among both Gram-negative bacteria and *S. aureus* isolates. Based on the European Centre for Disease Prevention and Control (ECDC) guidelines, the prevalence of MDR strains was as follows: *E. coli* (36%), *K. pneumoniae* (10%), *P. aeruginosa* (7%), and *S. aureus* (27%), MDR was defined as resistant to at least one agent in (≥ 3) antimicrobial categories.

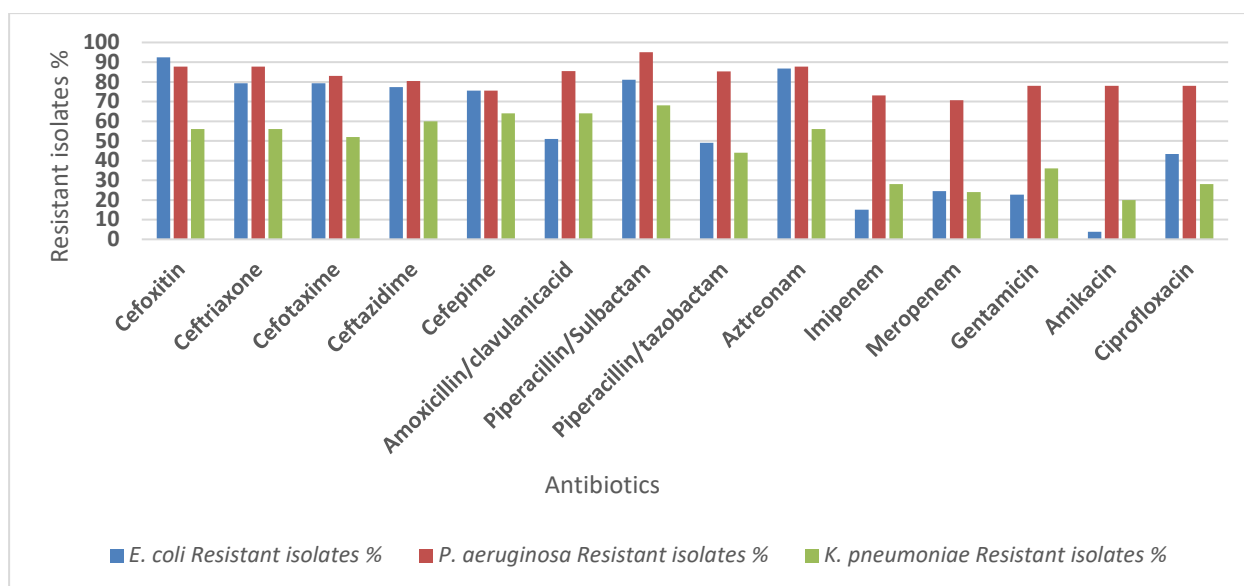


Fig. 4. Antibiotic resistance pattern of the isolated Gram-negative bacteria

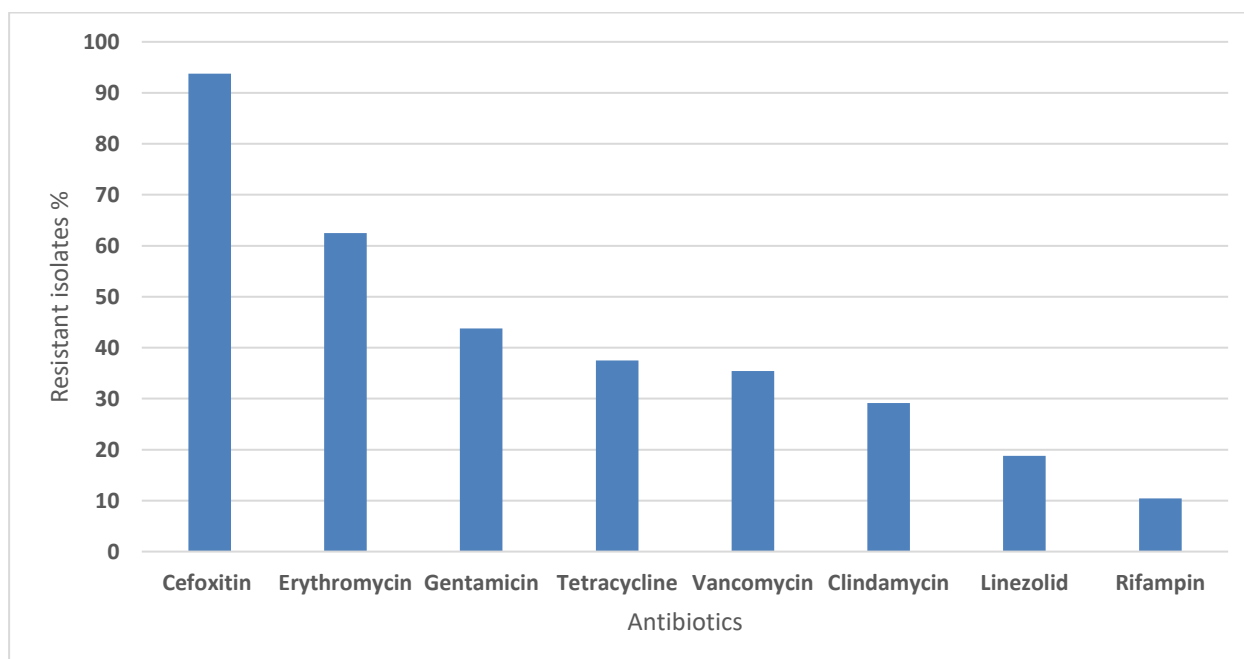


Fig. 5. Antibiotic resistance pattern of the isolated *S. aureus*

3.4.2 Agar well diffusion assay for purified flavonoids

The estimation of the antibacterial activity of flavonoids (F1 and F2) was studied against *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *S. aureus* using the agar well diffusion assay. The average of the inhibition zone around the wells containing F1 and F2 for represent strains shown in Table 4 and Figure 6. Among the examined bacteria, *P. aeruginosa* showed less sensitivity with a minimum inhibition zone 27.7mm for F1 and 24.9 for F2 at 125 µg/well. *E. coli* and *K.*

pneumonia showed maximum zone of inhibition of 29.7, and 29.8 mm for F1, 25.6 and 26.8 mm for F2, respectively. *S. aureus* isolates exhibit more or less similar zone of inhibition of 29.6 and 24.4 mm for F1 and F2 respectively.

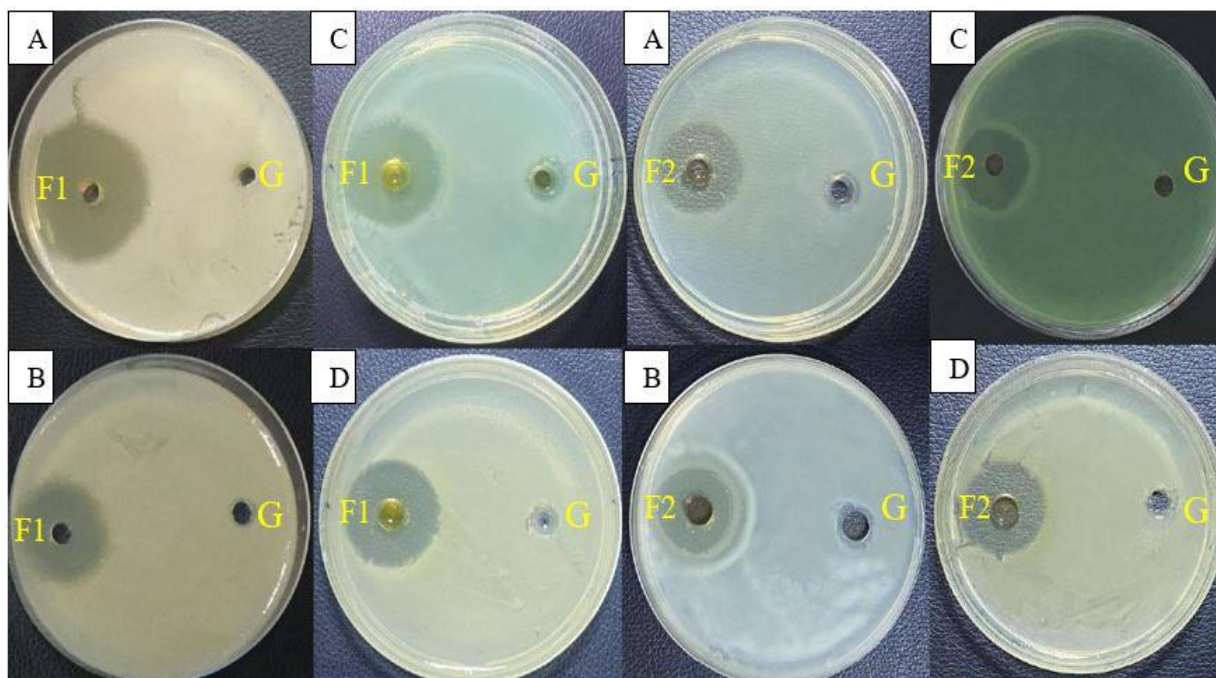


Fig. 6. Agar well diffusion assay on Mueller-Hinton agar showing the zones of antibacterial inhibition by flavonoids (F1 and F2) against *E. coli* (A), *K. pneumoniae* (B), *P. aeruginosa* (C), and *S. aureus* (D). Gentamicin (G) was used as a positive control, as all tested isolates were resistant to this antibiotic.

Table 4. The average of growth inhibitory zone (mm) of flavonoids (F1 and F2) against *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *S. aureus*.

Flavonoids	Pathogen growth inhibition zone (mm)			
	<i>E. coli</i>	<i>K. pneumonia</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
125 µg/well	NO=53	NO= 25	NO=41.	NO= 48
F1	29.7	29.8	27.7	29.6
F2	25.6	26.8	24.9	24.4

No= number of isolates

3.4.3 MIC and MBC of flavonoids

Flavonoid exhibited high efficacy against the tested pathogens. Table 5 presents the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of flavonoids (F1 and F2) and gentamicin against two multidrug-resistant (MDR) isolates of each species of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and methicillin-resistant *S. aureus* (MRSA). Flavonoid F1 exhibited the highest antibacterial effect, with significantly lower MIC and MBC values across all tested bacteria compared to flavonoid F2 and gentamicin. The lowest MIC for F1 was 7.8 µg/mL against *E. coli* (B7), followed by 15.62 µg/ml against *E. coli* (U21), *K. pneumoniae* (U5), *K. pneumoniae* (S11), *S. aureus* (U39), and 31.25 µg/ml against *P. aeruginosa* (S10), *P. aeruginosa* (S53), and *S. aureus* (S69). For F2, the MIC was 62.5 µg/ml against all tested bacteria. Gentamicin showed an MIC of 62.5 µg/ml against *E. coli* (B7), while the remaining isolates exhibited resistance at concentrations greater than 125 µg/ml.

MBC values for flavonoids are also summarized in Table 5. For F1, the lowest MBC (15.62 µg/mL) was observed against *E. coli* (B7). An MBC of 31.25 µg/ml was recorded for *E. coli* (U21), *K. pneumoniae* (U5), *K. pneumoniae* (S11), and *S. aureus* (U39), while 62.5 µg/ml was required for *P. aeruginosa* (S10), *P. aeruginosa* (S53), and *S. aureus* (S69). F2 showed an MBC of 125 µg/ml against all tested bacteria. All tested organisms were resistant to gentamicin, with MBC values exceeding 125 µg/ml, except for *E. coli* (B7) was <125 µg/ml.

Table 5. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of flavonoid (F & F2) and gentamicin (G) against eight isolates of multidrug-resistant bacteria.

Pathogen	Antimicrobial agent µg/ml					
	F1		F2		G	
	MIC	MBC	MIC	MBC	MIC	MBC
<i>E. coli</i> (B7)	7.8	15.62	62.5	125	62.5	125
<i>E. coli</i> (U21)	15.62	31.25	62.5	125	>125	>125
<i>K. pneumoniae</i> (U5)	15.62	31.25	62.5	125	>125	>125
<i>K. pneumoniae</i> (S11)	15.62	31.25	62.5	125	>125	>125
<i>P. aeruginosa</i> (S10)	31.25	62.5	62.5	125	>125	>125
<i>P. aeruginosa</i> (S53)	31.25	62.5	62.5	125	>125	>125
<i>S. aureus</i> (U39)	15.62	31.25	62.5	125	>125	>125
<i>S. aureus</i> (S69)	31.25	62.5	62.5	125	>125	>125

3.4.4 Effects of flavonoid (F1) on bacterial growth kinetics

Various concentrations of flavonoid (F1) were tested to examine its effects on the growth kinetics of eight multidrug-resistant (MDR) isolates: *E. coli* (U21, B7), *K. pneumoniae* (U5, S11), *P. aeruginosa* (S10, S53), and *S. aureus* (U39, S69). Growth was monitored by measuring optical density (OD) at 600 nm at different intervals using a microplate reader. All tested isolates, excluding the control group, were treated with two-fold serial dilutions of F1.

The results demonstrated the concentration-dependent inhibitory effects of F1 on bacterial growth. Noticeable inhibition was observed at 0.25 and 0.5 MIC (approximately 5–10%), significant inhibition at the MIC concentration (approximately 90%), and complete inhibition at 2X and 4X MIC (Fig. 7). All tested organisms were resistant to gentamicin at a high concentration (125 µg/ml), except for *E. coli* (B7), which exhibited resistance at 31.25 µg/ml.

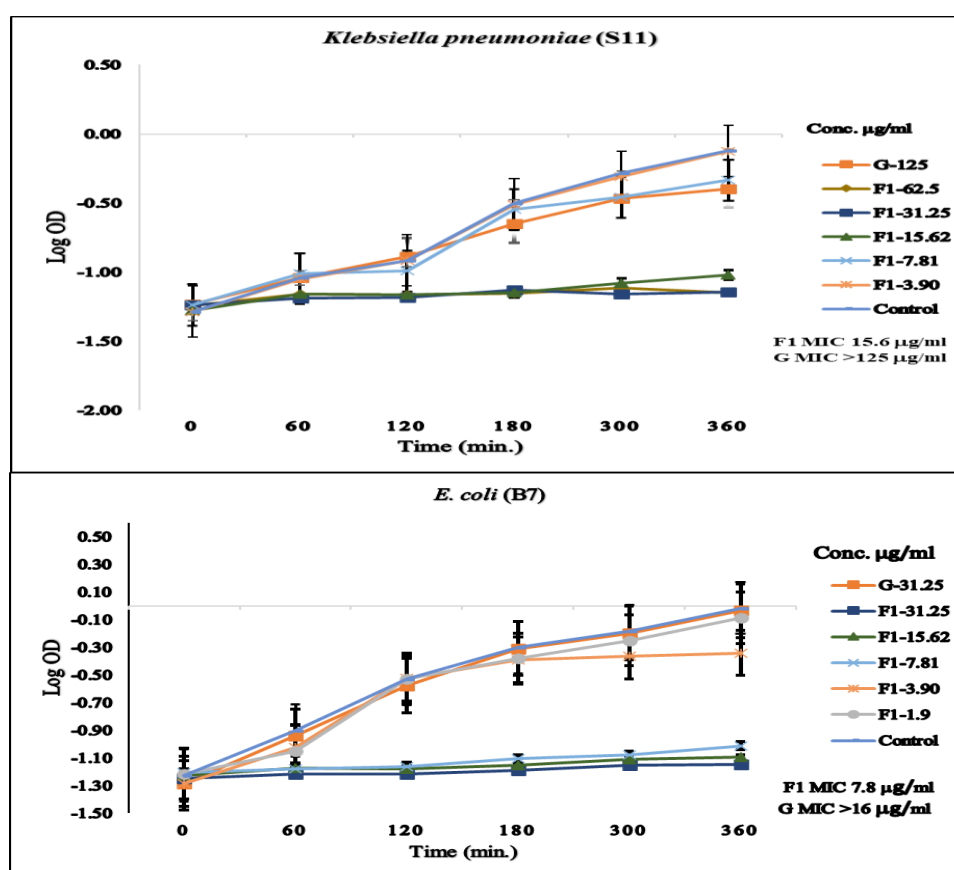


Fig. 7. Effects of flavonoid (F1) on the growth kinetics of *E. coli* (B7) and *K. pneumoniae* ((S11): The isolates were exposed to two subinhibitory and two suprainhibitory concentrations of flavonoid (0 MIC as control, 0.25 MIC, 0.5 MIC, MIC, 2X MIC, and 4X MIC) for 6 hours. The growth curves were dependent on flavonoid concentrations, with complete inhibition observed at a concentration of 2X MIC. Gentamicin (G) was used as a resistance control.

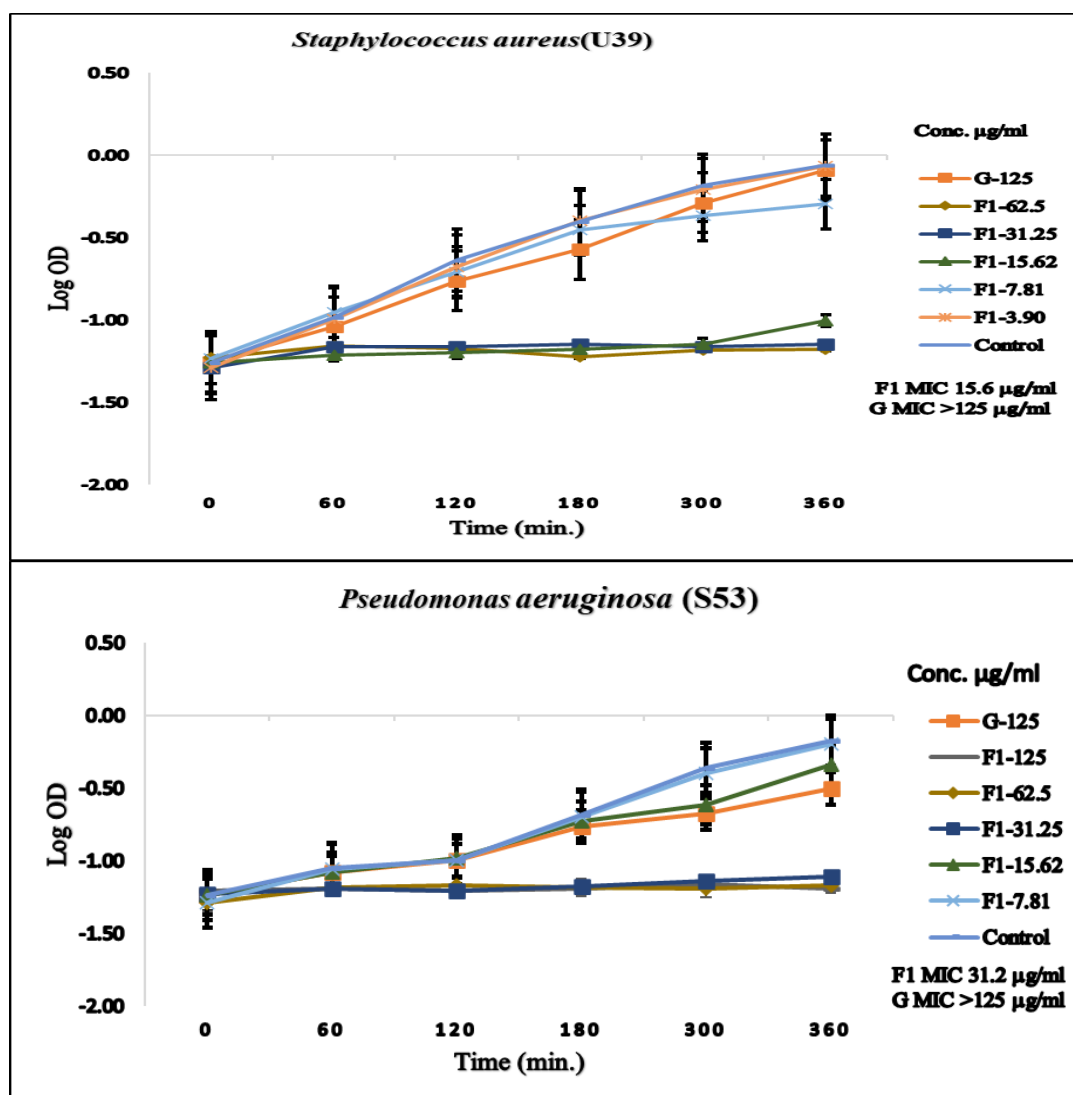


Fig. 7. Continued. Effects of flavonoid (F1) on the growth kinetics of *P. aeruginosa* (S53), and *S. aureus* (U39):

4. Discussion

4.1. According to phenotypic identification, *Saccharomyces cerevisiae* and *Sporobolomyces roseus* were detected, each with a frequency of 20%, out of all five soil samples.

4.2 Extraction, purification and identification of fungal flavonoids

Fungal flavonoids are diverse secondary metabolites produced by various fungi, including *Aspergillus*, *Penicillium*, and *Fusarium*, and possess antimicrobial, antioxidant, and other therapeutic properties [23, 24, 25]. These compounds, including flavonols and flavones, are found in fungal mycelia, spores, and fermentation broths [28]. Extraction of yeast flavonoids can be complicated because of their low abundance and the complexity of their chemical contents [29].

The successful application of 70% acetone as an extraction solvent demonstrates its efficacy in extracting flavonoids due to the ability to dissolve both polar and non-polar molecules [30,31]. The effective separation of the extracted compounds by use of TLC with an acetone:benzene (1:2) and the variations in R_f values reflect differences in polarity and molecular structure among the separated constituents, visualized via iodine vapor, which interacts with compound functional groups [18,32]. F1 and F2 were further purified by column chromatography, with TLC confirming their purity at R_f values of 0.97 and 0.87, respectively, demonstrating efficient separation based on molecular properties [33].

Organic compounds were identified using UV absorbance spectroscopy and Fourier-transform infrared (FTIR) spectroscopy. Both F1 and F2 fractions exhibited polar flavonoid characteristics with similar expected basic backbone structures. Comparable techniques have been utilized in previous studies for the detection of flavonoids, as demonstrated by Mabasa *et al.*, [34]. Additionally, Feng *et al.*, [35] reported specific UV absorbance spectra for various flavonoid subclasses: 254–280 nm or 340–360 nm for flavones and flavonols, 520–540 nm for anthocyanidins, and 250 nm for chromones. FTIR analysis align with previous reports and confirm the presence of flavonoid-specific structures and functional groups [36].

4.3 Antimicrobial activity of the extracted flavonoids

The extracted yeast flavonoid (F1 and F2) showed high antibacterial effects against MDR-tested bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*). The antibacterial activity of both compounds (F1 and F2) was investigated using agar diffusion and broth microdilution methods. The F1 agent showed larger inhibition zone diameter and lower MIC and MBC than the F2 agent. The reason for this may be the difference in the chemical structure or the size of the two compounds.

Also, the MIC and MBC values were relatively varied among test strains. This may be due to the difference in intrinsic resistance mechanisms used by these bacteria. The antibacterial activity of the purified flavonoids was further confirmed by studying their effect on bacterial growth kinetics at different concentrations. There was approximately 95% growth inhibition for all tested strains at 31.25 µg/ml of F1 agent. The results demonstrated the concentration-dependent inhibitory effects of F1 on bacterial growth. The results showed that the level of growth inhibition depended on the concentration of F1.

Previous studies have reported the antibacterial activities of flavonoids. Wu *et al.* [37] demonstrated high efficiency of different flavonoids against *S. aureus* (MRSA) with MIC

ranging from 2 μ g/mL to >128 μ g/mL, and *E. coli* with MIC >128 μ g/mL. Wang *et al.* [38] recorded MICs of 512 μ g/mL of flavonoids against *E. coli* and 1024 μ g/mL against *K. pneumoniae*. Stanley *et al.*, [39] mentioned that the MIC was 8 μ g/mL against different *P. aeruginosa* strains.

Comparing with the above studies, the results obtained in the present study showed that the efficacy of the extracted yeast was more effective against MDR bacteria, with a reliable value of MIC.

Numerous studies have investigated the antibacterial effects of flavonoids against both gram-positive and gram-negative bacteria. Flavonoids offer a promising solution to combat bacterial infections due to their antimicrobial action, which operates through mechanisms distinct from those of conventional drugs [40]. Structural modifications of flavonoids, such as hydroxylation (at C5, C7, C3', and C4), geranylation, or prenylation (at C6), have been extensively studied to enhance their antibacterial activity. However, methoxylation (at C3' and C5) has been found to diminish the antimicrobial effects of flavonoids [41]. FTIR analysis of F1 and F2 confirmed the presence of hydroxyl (O–H) and carbonyl (C=O) groups, which are key for antibacterial activity, these functional groups most likely increase bacterial membrane rupture and enzyme inhibition, which explains the reported antibacterial actions [36].

The structural features of flavonoids facilitate their interaction with lipid bilayers through two primary mechanisms. Firstly, flavonoids can partition into the hydrophobic interior of the membrane, where non-polar substances are accommodated. Secondly, they can form hydrogen bonds at the interface between the polar heads of lipids and the hydrophilic regions of flavonoids [42]. Moreover, flavonoids' non-specific interactions with phospholipids may induce structural alterations in the membrane, thereby indirectly affecting the organization and function of membrane proteins [43].

Overall, these findings underscore the potential of flavonoids as alternative antibacterial agents, particularly for tackling MDR pathogens. Their structural versatility and unique interaction pathways with bacterial membranes suggest a promising avenue for the development of flavonoid-based antimicrobials.

5. Conclusion

This study successfully extracted, purified, and characterized phenolic compounds (F) from the yeasts *Saccharomyces cerevisiae* and *Sporobolomyces roseus*. The findings highlight the potential of these yeast-derived flavonoids as promising alternatives for treating antibiotic-resistant bacterial strains (MDR), encouraging further exploration of their possible clinical applications in managing infections caused by these pathogens. Further research is needed to explore their mechanisms of action, safety, and potential clinical applications for treating infections caused by multidrug-resistant (MDR) bacteria.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

No conflicts of interest to declare

Funding Statement

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استخلاص الفلافونويدات من فطر *Saccharomyces cerevisiae* و *Sporobolomyces roseus* وتقييم فعاليتها المضادة للبكتيريا

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المستخلص

هدفت الدراسة إلى استخلاص الفلافونويدات من خميرة *Saccharomyces cerevisiae* وخميرة *Sporobolomyces roseus* وتقييم نشاطها المضاد للبكتيريا ضد مسببات أمراض بكتيرية مختارة. تم استخلاص الفلافونويدات (F) من الخمائر *Saccharomyces cerevisiae* و (*Sporobolomyces roseus*) باستخدام الأستون بتركيز 70%، متبوعاً بعمليات الفصل والتنقية عبر تقنية كروماتوغرافيا الطبقة الرقيقة (TLC) وكروماتوغرافيا العمود. ثم تم تحديد المركبات باستخدام مطيافية امتصاص الأشعة فوق البنفسجية (UV) ومطيافية الأشعة تحت الحمراء بتحويل فورييه (FTIR). وأظهرت النتائج أن الفلافونويدات المنقاة النهائية سجلت نسبة 10% من الفلافونيد (F1) المستخلص من *Saccharomyces cerevisiae* و 8% من الفلافونيد (F2) المستخلص من *Sporobolomyces roseus*. كما أظهرت نتائج امتصاص الأشعة فوق البنفسجية و FTIR أن كلاً من العميلين F1 و F2 يمتلكان طبيعة قطبية مع هياكل أساسية متشابهة للفلافونويدات المنقاة. أظهر العميلان F1 و F2 أنشطة عالية مضادة للبكتيريا ضد جميع البكتيريا المختارة للاختبار (*Klebsiella pneumoniae*) ، *Escherichia coli* ، *Pseudomonas aeruginosa* ، والمكورات العنقودية الذهبية المقاومة للميثيسيلين). سجل العميل F1 أدنى نطاق للتركيز المثبط الأدنى (MIC) تراوح بين (7.8–31.25 ميكروغرام/مل)، بينما سجل F2 تركيزاً مثبطاً ثابتاً قدره 62.5 ميكروغرام/مل ضد جميع البكتيريا المختبرة. تشير هذه النتائج إلى أن الفلافونويدات المشتقة من الخميرة تمتلك إمكانات كعوامل بديلة مضادة للميكروبات لمكافحة البكتيريا المقاومة للمضادات الحيوية..

الكلمات المفتاحية: الفلافونويدات، *Saccharomyces cerevisiae*، *Sporobolomyces roseus*، البكتيريا متعددة المقاومة للأدوية (MDR).