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GC-MS Analysis and Biological Evaluation of *Glycyrrhiza glabra* Root Ethyl Acetate Extract

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ABSTRACT. Natural products are an important source of chemically diverse compounds with significant potential for drug discovery. *Glycyrrhiza glabra* L. is a medicinal plant known for its diverse pharmacological activities. This study investigated the phytochemical composition and antioxidant and antimicrobial activities of the ethyl acetate extract of *G. glabra* (GGR-EA). Phytochemical profiling by gas chromatography-mass spectrometry (GC-MS) identified glabridin (43.77%) as the predominant compound, followed by 4′-O-methylglabridin (8.76%). The extract's antibacterial activity was assessed against four common pathogens: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231 via the disc diffusion method. Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, yielding an IC50 of 79.98 ± 4.48 µg/mL. The total phenolic content and total flavonoid content of the extract were found to be 92.16 ± 2.86 mg GAE/g dry extract and 10.12 ± 0.31 mg QE/g dry extract. GGR-EA exhibited antimicrobial activity against all tested microorganisms. The predominance of glabridin and 4′-O-methylglabridin in the extract suggests that these constituents may contribute to its antioxidant and antimicrobial properties. Overall, the findings highlight the pharmaceutical potential of *G. glabra* extract as a promising source of bioactive phytochemicals.

INTRODUCTION

Medicinal plants provide essential bioactive compounds with significant pharmacological properties (Oliveira *et al*., 2025). These secondary metabolites exhibit potent antimicrobial and antioxidant activities (Suryanti *et al*., 2020). Rising antibiotic resistance necessitates the discovery of novel natural alternatives (Ekwueme et al., 2025). Synthetic antioxidants and conventional antibiotics are under increasing scrutiny for their efficacy (John, 2024).

Therefore, plant-derived extracts offer a sustainable reservoir for the development of new therapeutic agents. In Indonesia, one of the renowned medicinal plants is *Glycyrrhiza glabra* L., commonly known as licorice (akar manis in Indonesia)(Semenescu *et al*., 2024) . It belongs to the Fabaceae (Leguminosae) family (Hasan *et al*., 2021). Due to its long history of medicinal use, akar manis is regarded as one of the world's most valuable medicinal herbs. It contains approximately 400 phytochemicals, such as flavonoids, phenols, saponins, triterpenes, anthraquinones, and essential oils (Tang *et al*., 2015). Glycyrrhizin, a primary saponin, provides its characteristic sweet flavor, making it widely used in food, pharmaceutical, and cosmetic sectors (Wahab *et al*., 2021). In addition, licorice roots also contain significant amounts of flavonoids such as glabridin, liquiritigenin, and isoliquiritigenin, which exhibit strong antibacterial (Yang *et al*., 2016) and antioxidant activities (Dang *et al*., 2021).

Flavonoids found in *G. glabra* L. are classified into several groups, including flavanones, flavones, flavanonols, chalcones, isoflavones, and isoflavans. These bioactive compounds, such as glabridin and 4'-O-methylglabridin, play a vital role in the biological activity of plants, including combating oxidative stress and microbial infections (Dang *et al*., 2021). The presence of such bioactive compounds makes licorice an important candidate for natural drug development. Reactive oxygen species (ROS) are naturally generated during metabolic

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processes in the body (Liu *et al*., 2018). At controlled levels, ROS are crucial for maintaining cellular homeostasis and regulating immunological reactions (Bardaweel *et al*., 2018). However, excessive production of ROS triggers harmful processes that can contribute to many diseases, including cancer, cardiovascular disorders, and diabetes (Liu *et al*., 2018). Flavonoids are among the most potent natural antioxidants. These compounds are essential for neutralizing free radicals and protecting biological systems from oxidative damage (Bardaweel *et al*., 2018).

Moreover, *G. glabra* L. extracts effectively slow down the proliferation of Gram-negative and Gram-positive pathogenic bacteria. These biological activities are mainly attributed to triterpenoid saponins, particularly glycyrrhizin, as well as flavonoids such as glabridin and liquiritigenin. These metabolites physically disrupt bacterial cell membranes and halt essential enzymatic and metabolic pathways (Chen *et al*., 2024). Additionally, *G. glabra* L. yields phenolic compounds that function effectively as free-radical scavengers (Kazlauskaite *et al.*, 2023). These phenolics neutralize reactive oxygen species to prevent cellular damage during oxidative stress (Semenescu *et al*., 2024).

G. glabra L. also exhibits significant antifungal activity against *Candida albicans,* an opportunistic polymorphic fungus responsible for both mucosal and systemic infections, especially in immunocompromised individuals. The pathogenicity of *Candida albicans* is primarily due to its capability to transition from yeast to hyphal forms and to develop resistant biofilms (Seleem *et al*., 2016; Silva *et al*., 2017). Bioactive constituents of *G. glabra* L., such as glabridin, licochalcone A, and glycyrrhizin, have been shown to impair fungal cell membrane integrity, prevent ergosterol formation, and interfere with biofilm formation. Additionally, some of these compounds exhibit synergistic interactions with conventional antifungal agents, such as fluconazole, thereby enhancing their therapeutic efficacy (Ngwu *et al*., 2024; Seleem *et al.*, 2016)

Given the increasing prevalence of antifungal resistance, the ability of *G. glabra* L. metabolites to target key virulence factors of *C. albicans* underscores its promise as a source of novel plant-based antifungal therapeutics. These properties draw attention to the potential of *G. glabra* L. as a natural medicinal agent for the treatment of disorders linked to oxidative stress and infections.

Many phytochemical studies on *G. glabra* L. used methanolic and ethanolic extracts. Although extracts of G. glabra L. prepared with various solvents have been widely studied, GC-MS-based identification of the specific metabolites present in the ethyl acetate fraction and their relationship to bioactivity remain limited in the literature. This represents a genuine knowledge gap, since solvent polarity influences which compounds are extracted and their relative abundance, an effect that is particularly relevant for semi-polar flavonoids such as glabridin. Therefore, this study employed GC-MS to characterize the phytochemical profile of the ethyl acetate root extract of *G. glabra* L., together with the determination of total phenolic content, total flavonoid content, antioxidant activity, and antimicrobial activity.

RESEARCH METHODS

The plant material used in this study consisted of roots of *G. glabra* L., which were purchased from Pasar Gede, a traditional local market in Surakarta, Indonesia. A range of analytical-grade solvents, such as ethyl acetate, n-hexane, methanol, and dimethyl sulfoxide (DMSO), was used in this study. Reagents such as Folin-Ciocalteu, aluminum chloride ($AlCl_3$), and DPPH (2,2-diphenyl-1-picrylhydrazyl) were employed for phytochemical and antioxidant analyses, while quercetin and gallic acid served as reference standards. All chemicals were obtained from reputable suppliers, namely Sigma-Aldrich and Merck.

The equipment used included a Büchner funnel filtration apparatus, rotary evaporator, UV-Vis spectrophotometer UV-1240 (Shimadzu, Japan), GC-MS equipment (Thermo Scientific TRACE 1310 Gas Chromatograph coupled with ISQ 7610 Single Quadrupole Mass Spectrometer), Scan 500 HD automatic colony counter and inhibitory zone reader (Interscience), sterile Petri dishes (90 mm diameter), micropipettes, sterile filter paper discs, incubator, laminar airflow cabinet, autoclave, and digital balance. Four microbial strains were used to determine antimicrobial effectiveness. These targets included *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231. Muller-Hinton Agar (MHA) was used as the culture medium. Ketoconazole and chloramphenicol were used as standard antimicrobial agents for comparison.

Preparation of Sample

Dried roots of *Glycyrrhiza glabra* L. were purchased from Pasar Gede, Surakarta, Indonesia, and authenticated by the Biology Department, Universitas Sebelas Maret (UNS), Indonesia (Plant Identification

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Certificate No: UN27.9.6.4/Lab/2026). After being cleaned and allowed to air dry at room temperature, the roots were ground into a fine powder using a mechanical grinder. To achieve a consistent particle size, the powder was run through a 40-mesh sieve. Sequential maceration of *G. glabra* L. roots was performed using three different solvents. First, n-hexane extracted non-polar components. Next, ethyl acetate and methanol captured semi-polar and polar compounds. This systematic approach maximized the yield of various chemical constituents.

The extraction began by mixing 1 kg of powder with 2 L of n-hexane. The sample remained in the solvent for 48 hours. Periodic shaking ensured thorough contact between the powder and solvent. This step was necessary to remove fats and other low-polarity compounds. The mixture was filtered under vacuum using a Büchner funnel, and the filtrate was concentrated under reduced pressure using a rotary evaporator to obtain the n-hexane extract, which was stored in an amber glass bottle. The residual plant material was subsequently macerated with 2 L of ethyl acetate for 48 h at room temperature. The extract was filtered and concentrated using the previously described method to obtain the ethyl acetate extract, which was stored in an amber glass bottle. Finally, the remaining plant residue was macerated. The next step involved adding 2 L of methanol to the sample for 48 hours. The resulting extract was passed through filter paper. It was then concentrated by removing the solvent under reduced pressure. This procedure followed the same protocol used for the previous solvents. Sequential maceration maximizes recovery across polarity ranges; the methanol step completes the protocol. However, only *G. glabra* L. ethyl acetate root extract (GGR-EA) was analyzed due to its high yield and relevance.

GC-MS Analysis

The GC-MS of volatile compounds in GGR-EA was performed using an ISQ mass detector and TG-SQC. The capillary column used had dimensions of 30 m × 0.250 mm × 0.25 µm. During the analysis process, the carrier gas used was Helium. At the 20:1 split ratio applied in split mode, a sample of 1.0 µL was injected. A temperature of 250 °C at the injector inlet was maintained under stable conditions at a purge flow rate of 5.0 mL/min. At the start, the oven temperature was set to 60 °C for 2 min. The temperature was then ramped up to 190 °C at 3 °C/min and held for 1 min, followed by a further ramp to 280 °C at 5 °C/min and held for 5 min. During the process, the total run time was 69.39 min. A 70 eV electron ionization (EI) mode was set on the mass spectrometer during operation. The temperature of the transfer line was 300 °C, while the temperature of the ion source was maintained at 290 °C. A 50-350 m/z range was used for scanning to obtain mass spectra with a dwell time of 0.2 s. The NIST mass spectra library was used as a spectral source for comparison, and compounds with similarity indices greater than 85% were reported.

Total Phenolics Determination

The determination of the total phenolic content (TPC) was performed for GGR-EA. The method used was the Folin-Ciocalteu colorimetric assay. This was done by dissolving crude extract (500 g) in methanol (100 mL). A 1 mL aliquot was further diluted with 5 mL of methanol and then maintained at room temperature for 10 minutes. Subsequently, a pre-diluted Folin-Ciocalteu reagent solution (0.5 mL reagent in 0.5 mL distilled water) was prepared before addition to the reaction mixture. Afterward, a 2% (w/v) Na_2CO_3 solution (1 mL) was added, and the resulting solution was vortexed for 30 s. For the next half an hour, the reaction mixture was incubated at 25 °C in the dark. A UV-Vis spectrophotometer was used to measure the absorbance at 750 nm. Quantification of TPC was performed over a concentration range of (0 – 50 µg/mL), and a calibration curve was constructed with gallic acid. Total phenolic content was calculated using Equation 1.

$$\text{TPC} = \frac{\text{C} \times \text{V}}{\text{m}} \quad (1)$$

Total phenolics were calculated using Equation 1, where C was the concentration of phenols established from the gallic acid calibration curve (µg/ml), V represents the volume of the sample (mL), and m is the weight of the plant extract in grams (Suryanti *et al*., 2020).

Total Flavonoids Determination

The $AlCl_3$ colorimetric method was used to quantify the total flavonoid content of GGR-EA. Results were reported as mg QE/g. This unit represents milligrams of quercetin equivalents per gram of extract. Briefly, 500 mg of extract was combined with an $AlCl_3$ solution (5 mL of 0.1 M) and vortexed for 15 s. The reaction mixture was then incubated at 25 °C. Following incubation for 30 min, a UV-Vis spectrophotometer was used to record absorbance at 415 nm, with methanol serving as the blank. A calibration curve constructed with quercetin was

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used to calculate concentrations over the 0 – 500 µg/mL range. Total flavonoid content was calculated using Equation 2.

$$TFC = \frac{C \times V}{M} \quad (2)$$

where C represents the concentration of flavonoids determined from the calibration curve of quercetin in µg/ ml, V is the volume in mL, and M is the weight of plant extract in grams (Afzal and Rajesh, 2021).

Antioxidant Activity Determination

The antioxidant activity of GGR-EA was evaluated using the DPPH radical-scavenging method. Solutions of GGR-EA at concentrations of 50-300 µg/mL were prepared. For the assay, 1 mL of each sample concentration was transferred into test tubes, and 2 mL of methanol and 1 mL of DPPH (100 µg/mL) solution were added. Methanol served as a blank. Absorbance was recorded after incubation in the dark (for 30 min) at 517 nm. For this purpose, a UV-Vis spectrophotometer was used. For the calculation of radical scavenging activity, Equation 3 was used to determine a percentage inhibition (Sariwati et al., 2024).

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}} \times 100 \quad (3)$$

IC_{50} was derived from a linear regression plot of % inhibition (y-axis) against concentration (x-axis); the concentration at y = 50% was taken as the IC_{50} (Azim et al., 2025).

Antimicrobial Activity Assay

Using the Disc diffusion method, the antimicrobial activity of GGR-EA was determined against four microbial strains. Microbial inocula were prepared by suspending fresh colonies in sterile saline and adjusting the turbidity to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). On 90-mm agar plates, the microbial suspensions were swabbed uniformly. At concentrations of 40, 60, 80, and 100 mg/mL, the test solution of crude GGR-EA was used to infuse sterile discs, which were then positioned at precise intervals on the agar medium. 1% DMSO was used as a negative control to verify that the observed activity was attributable to the sample rather than the carrier. Chloramphenicol (30 µg/disc) and ketoconazole (2%) were utilized as positive controls. These drugs targeted bacteria and fungi, respectively. This step established a reliable basis for comparison. Bacteria were incubated at 37 °C for 24 hours. Fungal samples were maintained at 25 °C for 72 hours. The zones of inhibition were measured in millimeters using a Scan 500 HD (colony counter). This process quantified the effectiveness of the treatment against each strain.

RESULTS AND DISCUSSION

GC-MS analysis of GGR-EA identified 48 peaks (Figure 1). Glabridin was the most abundant compound at 43.77%. This value exceeds the 16.38% reported by Mishra *et al*. (2021). The second major compound was 4′-O-methylglabridin (8.76%). This also surpasses the 4.1% found by Mishra *et al*. (2021). Full GC-MS characteristics are summarized in Table 1. Chemical structures of major bioactive compounds identified by GC-MS are shown in Figure 2.

Table 1. Identified compounds of GGR-EA by GC-MS analysis.

Retention Time (min)	Compound Name	Area (%)	Molecular Formula	Similarity Index
58.42	Glabridin	43.77	$C_{20}H_{20}O_4$	90%
56.91	4'-O-methylglabridin	8.76	$C_{21}H_{22}O_4$	87%

The GGR-EA contained 92.16 ± 2.86 mg GAE/g of phenolic compounds, which is considered moderate to high compared to other reported values for *G. glabra* extracts, such as 34.19 mg GAE/g (Zhou *et al*., 2019) and 169.83 mg GAE/g (Semenescu *et al.*, 2024), suggesting meaningful antioxidant and antimicrobial potential. This is consistent with GC-MS findings, where glabridin (43.77%), a polyphenolic isoflavonoid with phenolic hydroxyl groups capable of radical scavenging, was identified as the dominant compound. Many studies have described correlations between TPC and antioxidant activity in various plants (Bardaweel *et al.*, 2018; Semenescu *et al*., 2024). However, high phenolic concentrations do not necessarily lead to improved radical-scavenging performance, and this relationship is not necessarily linear. Several factors influence total phenolic content in plant

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extracts, including the timing of material collection, the specific plant part used, and the temperature and geographic characteristics of the plant's growing environment.

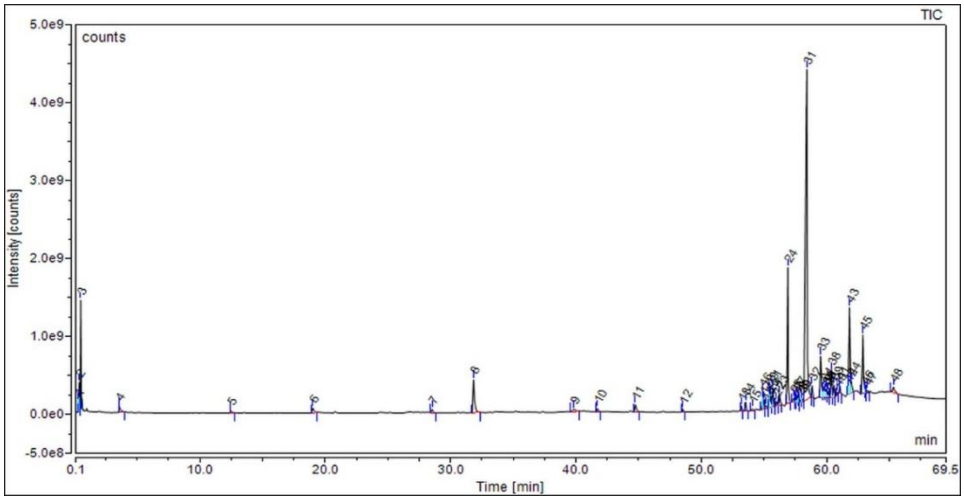
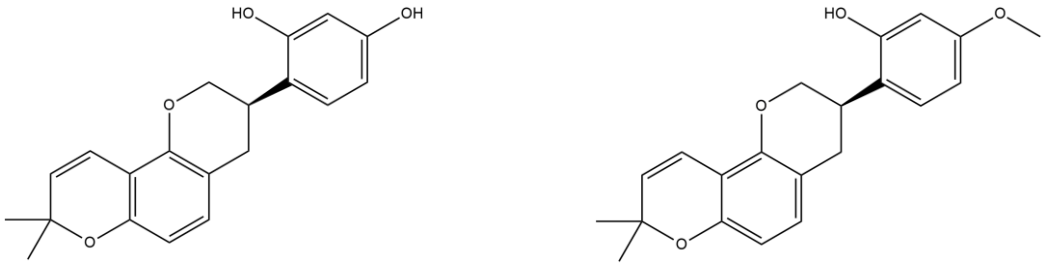


Figure 1. GC spectrum of GGR-EA.



(a) (b)

Figure 2. Structures of bioactive compounds from GGR-EA (a): Glabridin and (b) 4′-O-methylglabridin.

The total flavonoids in GGR-EA were calculated to be 10.12 ± 0.31 mg QE/g, indicating the presence of flavonoid compounds. This finding agrees with the GC-MS results. Glabridin and 4′-O-methylglabridin were identified in the extract as major compounds. Flavonoid constituents such as glabridin, liquiritigenin, and isoliquiritigenin are responsible for the biological functions of the plant. The effects include both antimicrobial and antioxidant actions (Babich *et al.*, 2022; Wahab *et al.*, 2021). The obtained value may differ from those reported in other studies due to variations in the extraction solvent, the plant part used, and environmental conditions, as flavonoid yield is highly dependent on these factors. These results support the therapeutic potential of *G. glabra* L., as flavonoids can exhibit strong pharmacological activity due to their high bioactivity and synergistic effects (Wu *et al.*, 2022).

The DPPH assay determined the antioxidant activity of GGR-EA and yielded an IC_{50} of 79.98 ± 4.48 µg/mL. This may be attributed to glabridin, the major compound identified by GC-MS (43.77%). According to the antioxidant activity classification reported by Kusumawati *et al.* (2021) an IC50 in the range of 50 – 100 µg/mL falls in the strong category; therefore, the value obtained in this study indicates strong antioxidant activity. The observed potency demonstrates stronger activity than previously reported values for *G. glabra* L. root extracts. A study showed DPPH IC_{50} (µg/mL) 116.17 for the methanolic extract of *G. glabra* L. roots (Zhou *et al.*, 2019) and an IC_{50} 385.85 µg/mL in another study (Semenescu *et al.*, 2024). These findings indicate that the antioxidant activity may be driven by selective, highly active compounds and their synergistic interactions rather than total phytochemical content.

GGR-EA showed antibacterial activity against all tested species, with activity increasing with increasing concentration of the crude extract. Studies show that plant extracts are often tested at high concentrations (e.g., 125 – 500 mg/mL) to produce inhibition zones (Mossie, 2024). The antimicrobial activity of GGR-EA may be attributed to the compounds identified by GC-MS. Glabridin (43.77%), the major constituent, is known to disrupt microbial cell membranes (Chen *et al.*, 2024); 4′-O-methylglabridin may exert its effects through similar mechanisms. Shinpterocarpin can inhibit cell wall synthesis (Batiha *et al.*, 2020). The lower susceptibility of

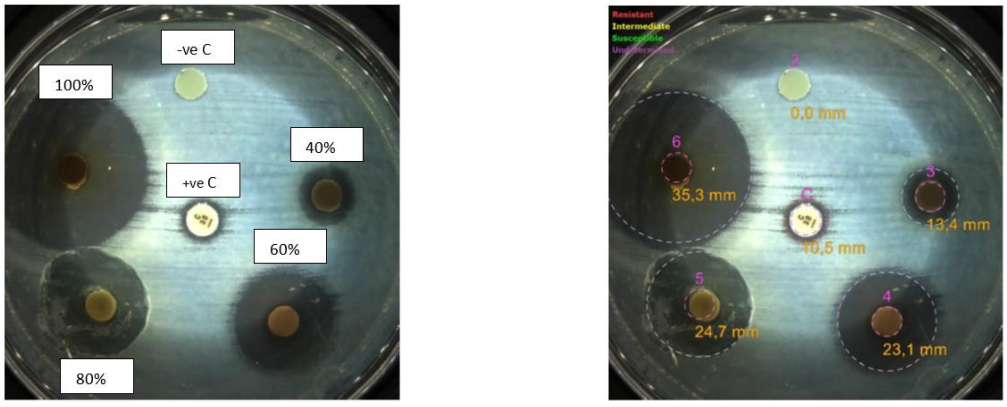
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Candida albicans compared to bacteria may be related to differences in cell envelope structure. The measured zones of inhibition are shown in Table 2.

Table 2. Inhibition zone (mm) of GGR-EA at various concentrations against microbes and positive and negative controls.

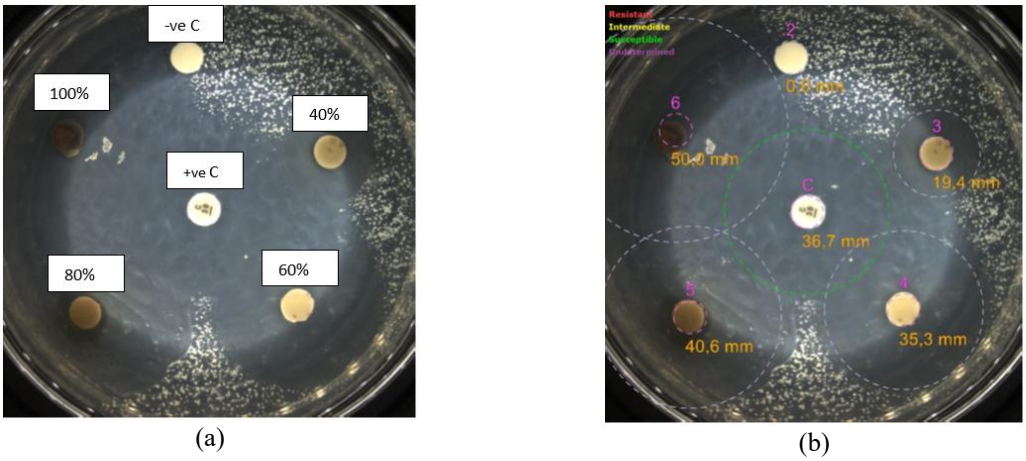
Species of Bacteria/ Fungi	Negative Control	40 mg/mL	60 mg/mL	80 mg/mL	100 mg/mL	Positive Control
P. aeruginosa	0.0	13.4	23.1	24.7	35.3	10.5 (chloramphenicol)
S. aureus	0.0	19.4	35.3	40.6	50.0	36.7 (chloramphenicol)
E. coli	0.0	14.0	23.7	25.6	31.3	36.2 (chloramphenicol)
C. albicans	0.0	0.0	9.3	9.6	25.6	34.5 (ketoconazole)

GGR-EA showed higher antibacterial activity against *P. aeruginosa* than the tested control, even at 40 mg/mL (Figure 3). On the other hand, in a previous study, no activity was observed even at a concentration of 100 mg/mL of the ethanolic extract, but inhibition zones of 9.67 mm and 11.8 mm were observed at concentrations of 400 and 600 mg/mL, respectively (Eshghi *et al*., 2019). Moreover, GGR-EA showed the highest antibacterial activity against *S. aureus* (Figure 4) at 100 mg/mL, with an inhibition zone of 50 mm, which is even higher than that of chloramphenicol. In a previous study, an inhibition zone of 25.1 mm was reported for *G. glabra* L. at 100 mg/mL of 80% ethanol extract against *S. aureus* (Sedighinia *et al*., 2012).



(a) (b)

Figure 3. The antibacterial activity of GGR-EA against *P. aeruginosa* before (a) and after analysis (b) using a Scan 500 HD.



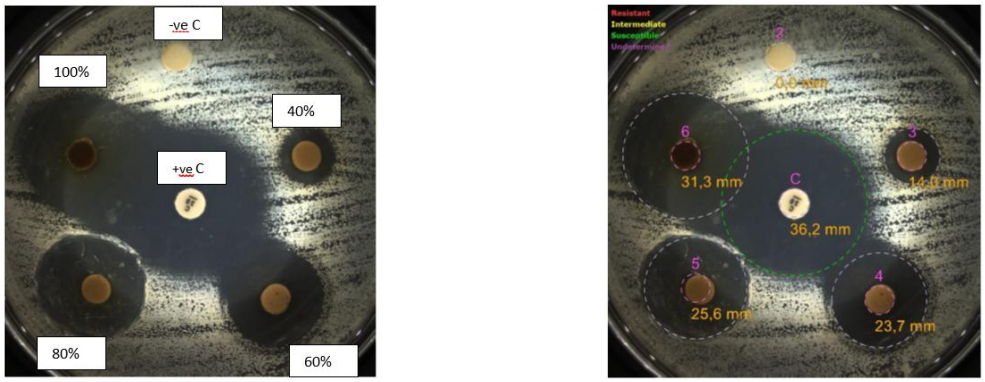
(a) (b)

Figure 4. The antibacterial activity of GGR-EA against *S. aureus* before (a) and after analysis (b) using a Scan 500 HD.

Furthermore, GGR-EA showed strong activity against *E. coli*, with an inhibition zone remaining closer to the control at the highest concentration, as shown in Figure 5. In a previous study, it was observed to be 22.5 mm at 100 mg/mL of 80% ethanol extract (Sedighinia *et al*., 2012), which is lower than the 31.3 mm observed at 100 mg/mL in this study. On the other hand, the fungal strain showed the lowest sensitivity, and the observed inhibition

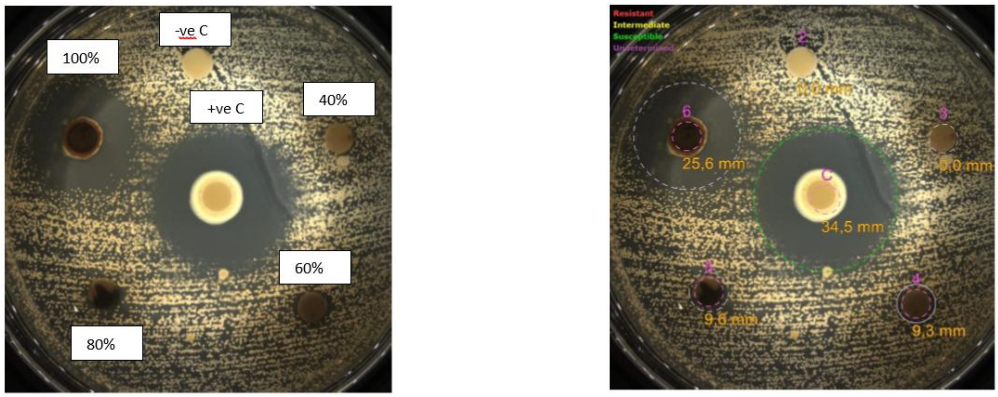
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zone was smaller than that of ketoconazole (Figure 6). In a previous study, *G. glabra* L. hydroalcoholic extract exhibited a 10 mm inhibition zone at a concentration of 50 mg/mL (Martins *et al.*, 2016), which is comparable to this study. However, the lower sensitivity of the fungal strain may be attributed to its ergosterol-based membrane and resistance mechanisms that reduce drug penetration (Seleem *et al.*, 2016). These results all indicate varying degrees of susceptibility among the tested pathogens.



(a) (b)

Figure 5. The antibacterial activity of GGR-EA against E. *coli* before (a) and after analysis (b) using a Scan 500 HD.



(a) (b)

Figure 6. The antibacterial activity of GGR-EA against *C. albicans* before (a) and after analysis (b) using a Scan 500 HD.

The significant antimicrobial activity observed in this study may be attributed to the presence of bioactive flavonoids identified in the extract. The major compound, glabridin (43.77%), has been widely reported to exhibit strong antimicrobial activity against a range of microorganisms. Similarly, 4′-O-methylglabridin, a derivative of glabridin, may also contribute to the observed antibacterial and antifungal effects due to its structural similarity and known bioactivity (Liu *et al*., 2018).

CONCLUSION

The study demonstrated that the ethyl acetate root extract of *Glycyrrhiza glabra* L. (GGR-EA) contained glabridin (43.77%) and 4′-O-methylglabridin (8.76%) as its predominant compounds. GGR-EA exhibited DPPH radical-scavenging activity with an IC_{50} of 79.98 ± 4.48 µg/mL and antimicrobial activity against all tested microorganisms. The lowest tested concentration showing antibacterial activity was 40 mg/mL against *P. aeruginosa*, *S. aureus*, and *E. coli*, whereas antifungal activity against *C. albicans* was observed at 60 mg/mL. These findings highlight GGR-EA as a promising natural source of bioactive phytochemicals for further pharmaceutical and nutraceutical research.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

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AUTHOR CONTRIBUTION

SK: Research Conceptualization and Design, Conducting Experiments, Data Collection, Laboratory Analysis, and Initial Manuscript Writing; VS: Methodology Guidance, Funding, Experimental Supervision, Data Validation, and Manuscript Revision for Scientific Quality; TK: Data Analysis, Review, Manuscript Editing.

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DECLARATION OF GENERATIVE AI

During the preparation of this work, the author used Grammarly to rephrase and improve the language of the manuscript. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the published article.

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