http://dergipark.org.tr/tr/pub/anatolianbryology

DOI: 10.26672/anatolianbryology.1705187

Anatolian Bryology
Anadolu Briyoloji
Dergisi
Research Article
e-ISSN:2458-8474
Online

Phytochemical profiling of *Brachythecium glareosum* (Bruch ex Spruce) Schimp. and preliminary *in silico* assessment of antifungal potential

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Received: 23 May 2025 **Revised: 17 June 2025** **Accepted: 18 June 2025**

Abstract

Phytochemical studies show that mosses can produce a range of bioactive chemicals that enhance defense mechanisms and ecological resilience. This study aims to investigate the phytochemical constituents and pharmacological potential of *Brachythecium glareosum* (Bruch ex Spruce) Schimp. to understand their bioactive potential and to stimulate future research into bryophyte-derived natural products. It also aims to be one of the first studies on *B. glareosum* in terms of phytochemicals and bioactivity. HPLC analysis revealed the presence of several phenolic compounds in the extract of *B. glareosum*. The study revealed the in silico antifungal potential of some phenolic compounds. It has been calculated that 2,5-Dihydroxybenzoic acid and 3,4-Dihydroxybenzoic acid compounds will inhibit Cytochrome P450 14Alpha-Sterol Demethylase (Cyp51) of Mycobacterium tuberculosis at the micromolar level. The study suggests that using more advanced methods to analyze B. glareosum could help find more useful compounds that can be used for treatment. Further biological assays are warranted to validate the antifungal activity indicated by in silico predictions.

Keywords: Bryophyte, Phytochemistry, Antifungal Activity, Phenolic Compounds, *In Silico*

Brachythecium glareosum (Bruch ex Spruce) Schimp. türünün fitokimyasal profillemesi ve *in silico* destekli antifungal potansiyelinin ön değerlendirmesi

Öz

Bu çalışma, *Brachythecium glareosum* (Bruch ex Spruce) Schimp.'un fitokimyasal bileşenlerini ve farmakolojik potansiyelini araştırmayı, biyoaktif potansiyellerini anlamayı ve briyofit türevi doğal ürünlerle ilgili gelecekteki araştırmaları teşvik etmeyi amaçlamaktadır. Ayrıca, fitokimyasal ve biyoaktivite açısından *B. glareosum* üzerine yapılan ilk çalışmalardan biri olmayı hedeflemektedir. HPLC analizi, *B. glareosum* özütünün bazı fenolik bileşiklerinin tespitini ortaya koymuştur. Bazı fenolik bileşiklerin *in silico* antifungal potansiyeli ortaya çıkarılmıştır. 2,5-Dihidroksibenzoik asit ve 3,4-Dihidroksibenzoik asit bileşiklerinin, *Mycobacterium tuberculosis*'in Sitokrom P450 14Alfa-Sterol Demetilaz'ını (Cyp51) mikromolar düzeyde inhibe ettiği hesaplanmıştır. Bu çalışma, *B. glareosum*'un fenolik profilinin daha geniş analitik tekniklerle genişletilmesinin terapötik açıdan önemli ek biyoaktif bileşikleri ortaya çıkarabileceğini öne sürmektedir. İn siliko tahminlerle belirtilen antifungal aktiviteyi doğrulamak için daha fazla biyolojik analiz yapılması gerekmektedir.

Anahtar kelimeler: Briyofit, Fitokimyasal, Antifungal Aktivite, Fenolik Bileşikler, *In Silico*

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To cite this article: *Çambay Z. Uslu H. Özdemir Bayçınar K. Göktaş B. Güngören M. Alataş M. 2025. Phytochemical profiling of Brachythecium glareosum (Bruch ex Spruce) Schimp. and preliminary in silico assessment of antifungal potential. Anatolian Bryology. 11:1, 44-51.*

1. Introduction

Bryophytes are a widely distributed and poorly studied group of plants worldwide, with an estimated number of 22,000-25,000 species (Asakawa and Ludwiczuk, 2013; Frahm, 2004). It is a moss belonging to the Bryophyta division, Bryopsida class, Hypnales order, Brachytheciaceae family. It is one of the 13 taxa of the *Brachythecium* genus in our country (Kürschner and Erdağ, 2021; Kürschner and Frey, 2011).

Bryophytes, including mosses, liverworts, and hornworts, are early land plants with remarkable biochemical diversity despite their relatively simple morphology. The genus *Brachythecium* (family: Brachytheciaceae) constitutes a prevalent and taxonomically diverse group among mosses (Ignatov and Huttunen, 2002). Species in this genus are increasingly being studied not just for their ecological services, but also for their potential and unique secondary metabolites. Some phytochemical investigations indicate that mosses can produce a range of bioactive chemicals, including phenolics, terpenoids, fatty acids, and sterols, which enhance their defensive mechanisms and ecological flexibility (Asakawa, 2007; Frahm, 2004).

Although the medical use of bryophytes seems to have been around for a few decades, these plants have been used as natural medicines for centuries, mostly in Asian countries (Asakawa, Tori, Masuya, and Frahm, 1990; Bodade, Borkar, Saiful, and Khobragade, 2008; Zinsmeister, Becker, and Eicher, 1991). Despite limited research, *Brachythecium* species have demonstrated potential antibacterial, antioxidant, and anti-inflammatory properties in preliminary screenings. In particular, *Brachythecium rutabulum* extracts exhibit inhibitory activity against prevalent bacterial and fungal pathogens, indicating their potential application in natural antimicrobial formulations. A study on methanolic extracts of 15 mosses showed that among the extracts *Brachythecium populeum* and *Brachythecium rutabulum* exhibit noteworthy antimicrobial potential, demonstrating inhibitory activity against bacteria including *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, as well as fungi such as *Aspergillus flavus*, *Candida albicans*, and *Trichophyton rubrum* (Kabadere, Birgi, Vatan-Öztopçu, İscen, and İlhan, 2021; Singh, Rawat, and Govindarajan, 2007). Furthermore, these extracts exhibit cytotoxic effects on human carcinoma cell lines and possess notable antioxidant properties (Ahmed et al., 2017; Klavina et al., 2015; Smolińska-Kondla et al., 2022). A comparative investigation on *Brachythecium populeum* revealed that the antioxidant activity (DPPH and ABTS) and the total polyphenol and flavonoid levels of the ethanol extract exceeded the values of the water extract by more than threefold. Furthermore, the MTT assay indicated that neither extract exhibited cytotoxicity. The anti-inflammatory potential of the ethanol extract was assessed by measuring the suppression of nitric oxide and inflammatory cytokine production (Park and Lee, 2023).

The pharmacological properties of *Brachythecium* species are highly relevant to their secondary metabolite composition. It has been reported that compounds such as flavonoids, triterpenoids and aromatic acids identified in different moss species have free radical scavenging, cell proliferation inhibitory and enzyme inhibitory properties (Rios and Recio, 2005). The lipid and sterol content of mosses contributes to their structural integrity and interactions with biological membranes, suggesting that these substances may exhibit cytotoxic or protective effects in pharmaceutical applications (Klavina et al., 2015). The LC-HRESIMS analysis of the methanolic extract performed by Elkhateeb and Daba revealed that *Brachythecium velutinum* included flavonoids (hyperoside, robinetin, and scutellarein) and terpenes (bufotalin, dantaxusin A, moreollic acid, taxuspines B and C, dihydroisomorellin, etc.). Additionally, *Brachythecium rutabulum* was found to contain sterigmatocystin (a xanthene molecule) and propinquanin B (a polyketide), both recognized for their cytotoxic properties, along with taxuspines B and C and schisantherin I. Furthermore in another study the HPLC results indicated that the phenolic acid content of the immature capsule extract of *Brachythecium rutabulum* comprised gallic acid, vanillic acid, and caffeic acid, whereas the shoot extract contained gallic acid and trans-cinnamic acid. Following the hydrolysis of the extracts, a significant quantity of ferulic acid was identified (Davidson, Harborne, and Longton, 1989; Elkhateeb and Daba, 2020).

This study presents a preliminary phytochemical screening of *B. glareosum*, aiming to identify key phenolic compounds and assess their potential antifungal interactions through computational docking analyses. To our knowledge, this is the first such study on this species.

2. Materials and Methods

2.1. Plant material and extraction process

The powdered plant was macerated with 70% ethanol for 3 days by rotating on a rotavapor for 1 hour. After filtering, it was evaporated to dryness on a rotavapor. The yield of the extract was determined as 8.50%. The research materials were collected from Amcabey Alabalık Surrounding, Ulukışla

(Niğde), located in the C13 (Figure 1). Square in Turkey according to the Henderson (Henderson, 1961) grid system. *B. glareosum*; It is a taxon that spreads in dry basic soils, rocks, coasts, grasses and open forest areas, and loves semi-arid and semi-neutral shade environments (Dierssen, 2001; Smith, 2004) (Table 1). The bryophyte specimen (*B. glareosum*) used in the study is preserved in the bryophyte collection of ALATAŞ (Tunceli) with the Herbarium number ALT 4632.

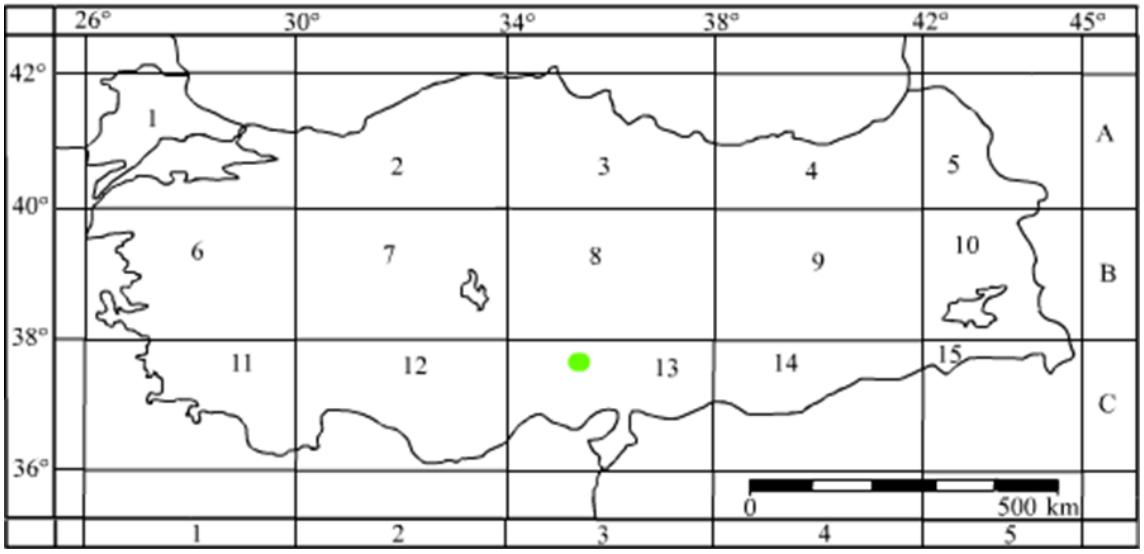


Figure 1. According to the Turkish grid system, the locality where the bryophyte sample was taken

Table 1. Locality information

Locality	Altitude (m)	Date	GPS Record
Amcabey Alabalık Surroundings, Ulukışla, Niğde (Green Dot)	1216	13.09.2024	34°41'28.80"E, 37°28'47.27"N

2.2. Phenolic content

HPLC grade methanol was purchased from J.T. Baker, Acetonitrile was purchased from Isolab and all other chemicals were purchased from Merck. 8.5mg (425 mg/ml) of the aboveground parts of *B. glareosum* was taken and closed in an airtight bottle. Alliance e2695 HPLC device and C18 (250x4.56mm.5µ) column were used to determine the phenolic compounds of the aboveground parts of *B. glareosum*. Phosphoric acid (pH 2) and Methanol (90): Acetonitrile (10) were used as mobile phases. The flow rate was set as 0.8 ml/min. The wavelength was determined as 280 nm and a Diode arrray detector was used. The injection volume was 20 µL and the temperature was 30°C. The results were taken with the Empower 3 program (Pirisi, Cabras, Cao, Migliorini, and Muggelli, 2000; Veneziani et al., 2018).

2.3. Molecular docking

The compounds detected in the phenolic components of *B. glareosum* were plotted to get SMILES from PubChem (https://pubchem.ncbi.nlm.nih.gov). The two substances found in the highest amounts in the extract were identified and the molecular docking process was applied to these substances. Energy minimization of two components were performed with the ChemOffice software. To evaluate 1EA1-antifungal activity, molecular docking studies were performed using the standard procedure to determine the binding modes and docking scores of the two compounds detected in *B. glareosum* extract two phenolic components at the active sites of the 1EA1 (Podust, Poulos, and Waterman, 2001). The macromolecule crystal structures was retrieved from the Protein Data Bank server (https://www.rcsb.org/, accessed 23 May 2025) and optimized with Schrödinger Maestro (Maestro, 2024). Molecular docking was performed with both Autodock and Autodock Vina programs (Eberhardt, Santos-Martins, Tillack, and Forli, 2021; Huey, Morris, Olson, and Goodsell, 2007). Since we had previously worked with this macromolecule for 1EA1, Fluconazole (Pdb ID: TPF) was re-docked into the target site of the macromolecule to validate the docking program and the RMSD value was found to be appropriate (<1) (Unver, Uslu, Gurhan, and Goktas, 2024).

3. Results and Discussion

3.1. Phenolic content

According to the results of the applied HPLC analysis, 7 phenolic components were determined

B. glareosum extract (Table 2). Two of these compounds were detected at very high concentrations compared to the others. 3,4-Dihydroxybenzoic acid was found at 16.897 ppm and 2,5-Dihydroxybenzoic acid was found at 6.848 ppm. Other compounds obtained were detected in the range of 2.335 ppm to 0.094 ppm (Table 2).

3.2. Molecular docking

The active binding sites of the macromolecule (Pdb ID: 1EA1) have been previously determined in the protein data bank (Podust et al., 2001). Docking studies were performed to see the interaction modes of the two phenolic compounds detected at the highest concentration in the *B. glareosum* extract with the active site of the macromolecule. Binding types and associated residues were generated in detail by Maestro Software (Table 3, Figure 2-5). Some residues previously identified as important for the interaction Mycobacterium tuberculosis in complex with fluconazole were described in detail in our previous study (Göktaş et al., 2024; Unver et al., 2024). The interaction modes with 1EA1 for 2,5-Dihydroxybenzoic acid and 3,4-Dihydroxybenzoic acid were visualized in 2D and 3D with the Maestro program (Maestro, 2024).

Table 2. Chemical compositions of the phenolic components of *B. glareosum* extract.

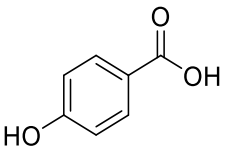
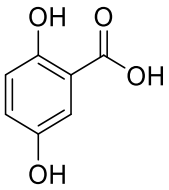
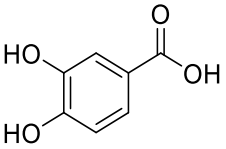
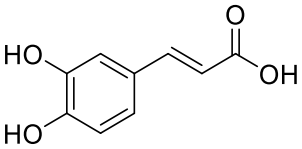
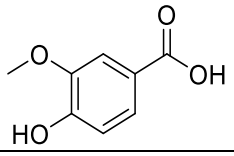
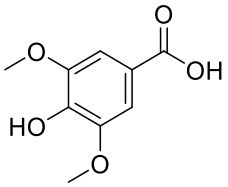
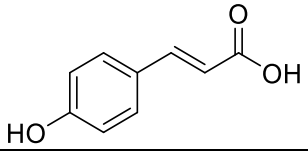
No	Compound	Chemical Structure	ppm	R^2
1	4-Hydroxybenzoic acid		0.681	0.999800
2	2,5-Dihydroxybenzoic acid		6.848	0.999979
3	3,4-Dihydroxybenzoic acid		16.897	0.997677
4	Caffeic acid		0.346	0.999713
5	Vanillic acid		2.335	0.999671
6	Syringic acid		1.266	0.983057
7	*p*-Qumaric acid		0.094	0.999736

Table 3. Molecular docking scores and estimated inhibition constants of Chemical compositions of phenolic components of *B. glareosum* extract and 1EA1.

Compounds	Autodock Results		Vina Results
	Estimated Inhibition Constant, Ki	Best Docking Score	Best Docking Score
2,5-Dihydroxybenzoic acid	88.91 µM	-5.53	-6.2
3,4-Dihydroxybenzoic acid	82.84 µM	-5.57	-6.3

µM: micromolar, Docking Score: Estimated Free Energy of Binding (kcal/mol)

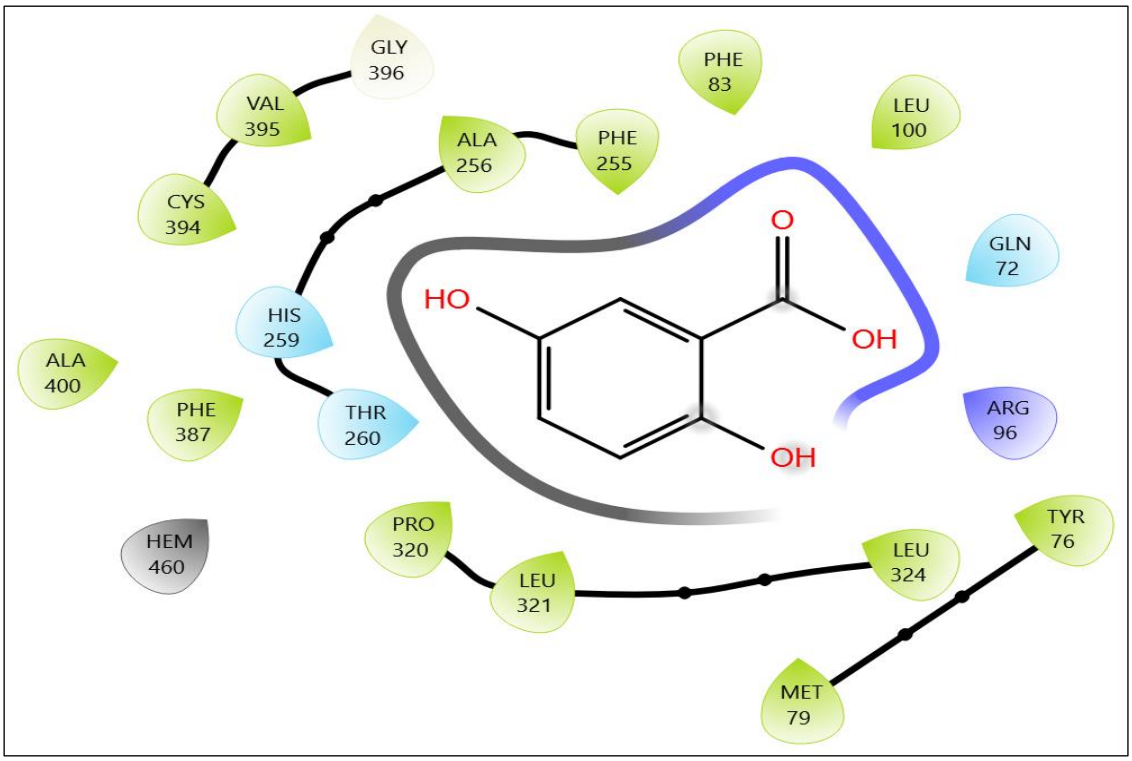


Figure 2. 2D interaction diagram with 1EA1 for 2,5-Dihydroxybenzoic acid.

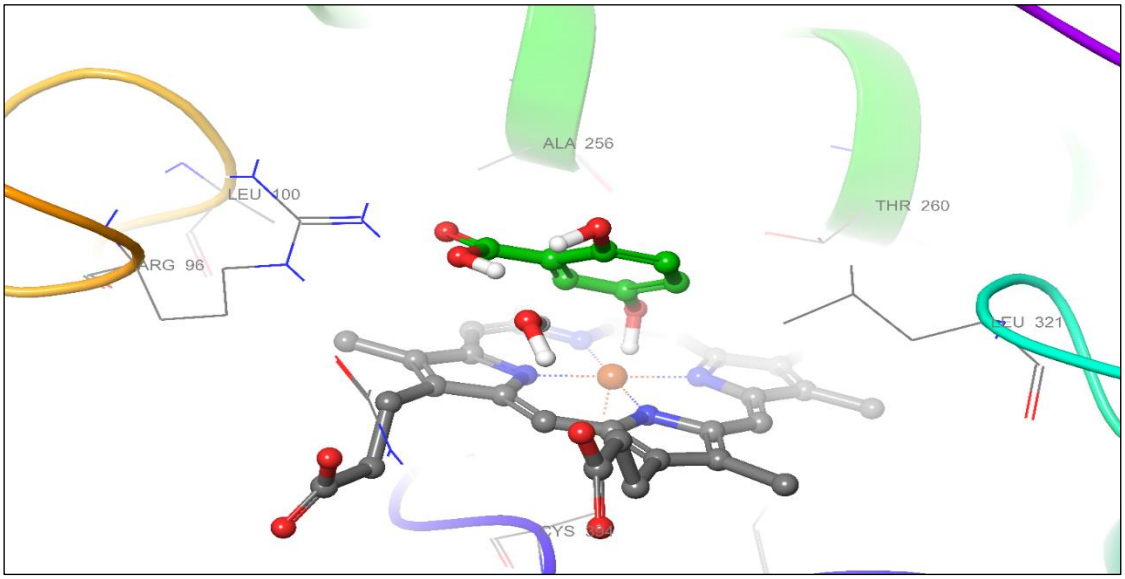


Figure 3. 3D interaction diagram with 1EA1 for 2,5-Dihydroxybenzoic acid.

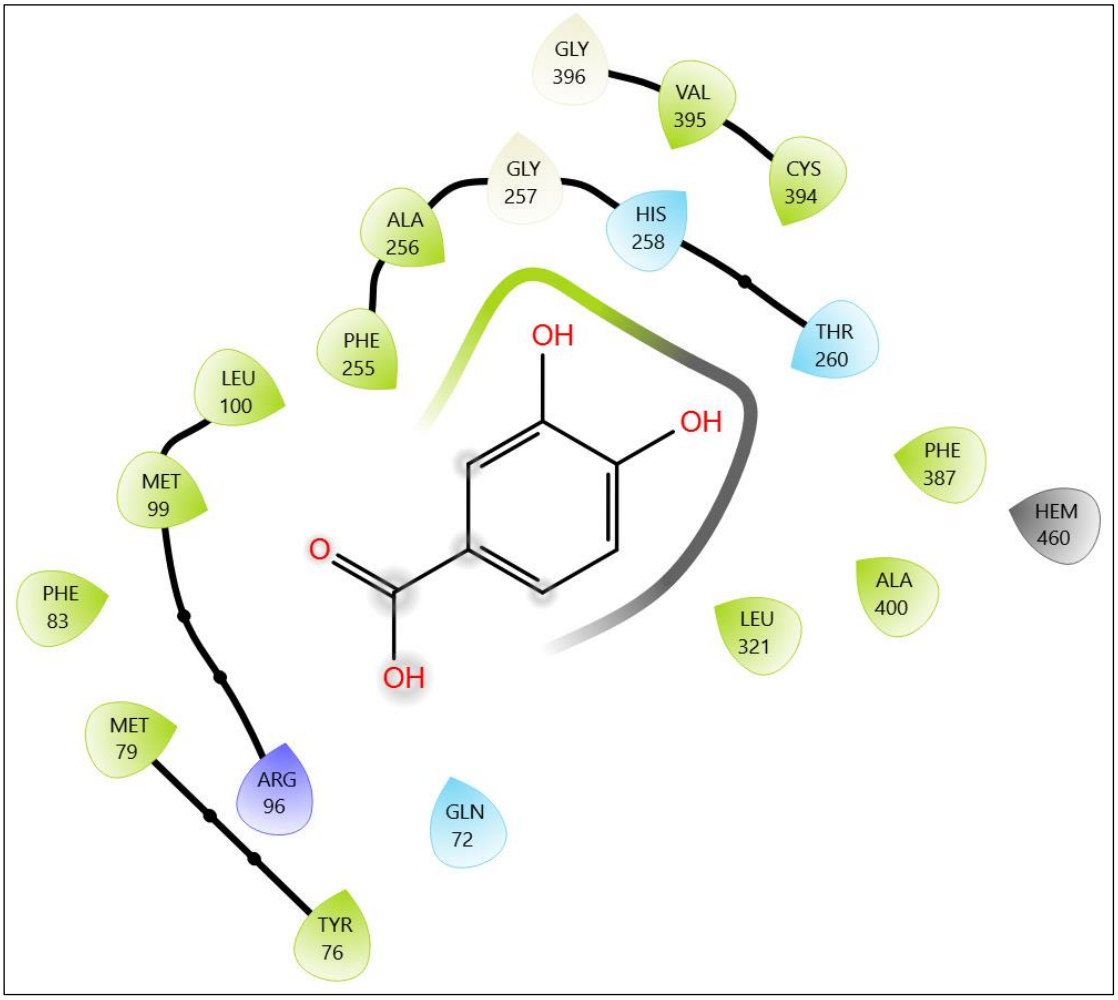


Figure 4. 2D interaction diagram with 1EA1 for 3,4-Dihydroxybenzoic acid.

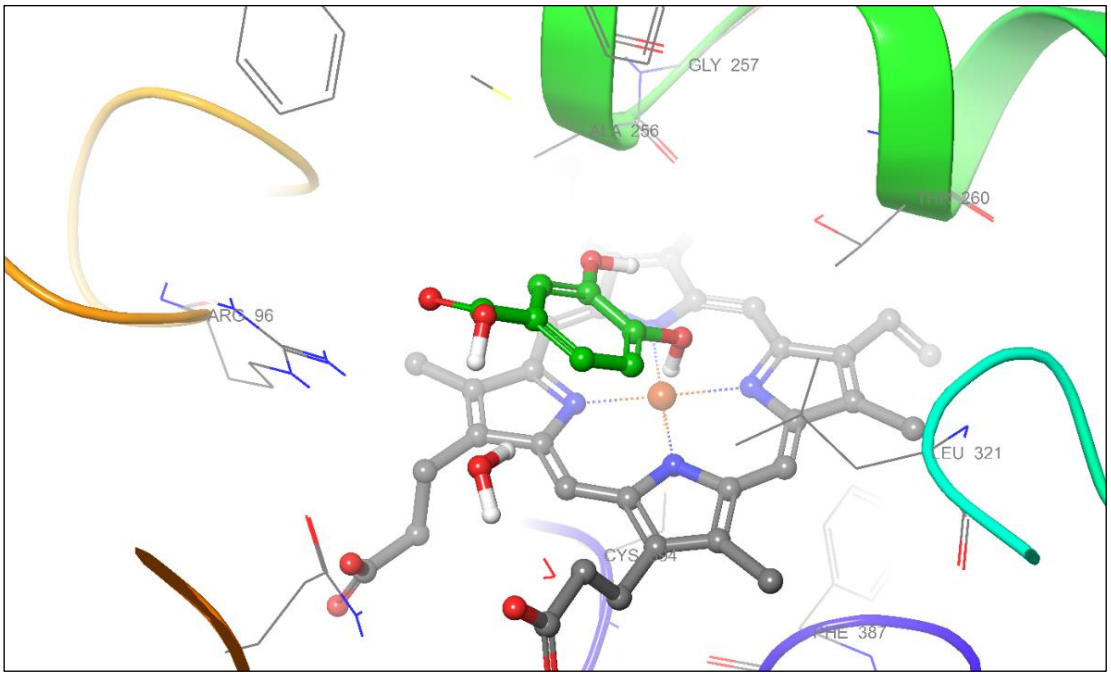


Figure 5. 3D interaction diagram with 1EA1 for 3,4-Dihydroxybenzoic acid.

In conclusion, this study was carried out to reveal the *in silico* antifungal potential of phenolic components of *B. glareosum* extract. HPLC analysis revealed the detection of some phenolic compounds. Molecular docking study revealed dock scores and interacting residues. Compounds 2,5-Dihydroxybenzoic acid and 3,4-Dihydroxybenzoic acid were computationally found to inhibit Mycobacterium Tuberculosis Cytochrome P450 14Alpha-Sterol Demethylase (Cyp51) at micromolar levels. Although molecular dock scores were good with 1EA1 selected for antifungal activity study, it was determined that no interaction such as hydrogen bonding, pi interaction was observed when the relevant images were examined. If the phenolic content determination can be expanded in terms of the number of compounds, antifungal activity research can be improved in this respect.

In conclusion, this study, in which some phenolic contents of *B. glareosum* were determined and its antifungal activity was investigated *in silico*, will constitute a resource for other studies to be conducted in the future.

Declaration

Author contributions: Concept, ZÇ; Conception and design, ZÇ, HU; Supervision consultancy, MA; Resources, BG, KÖB; Materials, MA, ZÇ; Data collection and/or processing, ZÇ, KÖB; Analysis and/or interpretation, MG, HU, BG; Literature search, ZÇ, KÖB, MA; Writing stage, ZÇ; Critical review, MA, ZÇ.

Conflict of interest: The authors have no competing interests to declare regarding the content of this article.

Funding: The authors declare that no funding, grant or other support was received during the preparation of this article**.**

Ethical approval: This research did not involve human or animal subjects and therefore does not require ethical approval.

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