ATTAHADI MEDICAL JOURNAL
Journal homepage:
http://attahadi.edu.ly/journal/index.php/amj/index

Evaluation of the Antimicrobial Effects of The *Genus Scrophulariaceae*

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Keywords.
Scrophulariaceae, Antimicrobial Activity, Microorganisms, Bacteria, Fungi.

Received *11 Mar 25*
Accepted *13 May 25*
Published *22 May 25*

A B S T R A C T
In this study, the antimicrobial activities of plants from the genus Scrophularia (family Scrophulariaceae) were evaluated. The specimens were extracted using a 60% alcohol solvent. These extracts were tested against various microorganisms, including Bacillus subtilis (DSMZ 1971), Candida albicans (DSMZ 1386), Enterococcus faecalis (ATCC 29212), and Staphylococcus epidermidis (DSMZ 20044). The disk diffusion (DD) method and the minimum inhibitory concentration (MIC) method were employed to assess antimicrobial activity. The highest activity was observed against Staphylococcus epidermidis, with an inhibition zone diameter of 11 mm, followed by Bacillus subtilis with an 8 mm zone diameter. The results suggest that Scrophularia extracts exhibit antimicrobial properties and could potentially serve as antimicrobial agents. However, further research is needed to explore their efficacy and applications more comprehensively.

Citation info. Alahmer N. Evaluation of the Antimicrobial Effects of The Genus Scrophulariaceae. Attahadi Med J. 2025;2(2):123-126. https://doi.org/10.69667/amj.25210

INTRODUCTION

The therapeutic potential of plants has significantly increased their use in treating various human disorders and infectious diseases caused by microorganisms. Research on plant extracts and derived products has demonstrated that higher plants possess potent antibiotic effects [1,2]. Medicinal plants harbor numerous phytochemicals that constitute the backbone of their defense mechanisms. These bioactive compounds are found in different plant parts—such as flowers, leaves, and roots—of vegetables and fruits, providing not only essential nutrients and fiber but also protection against diseases. Recent studies highlight the abundance of phytochemicals in medicinal plants, many of which serve as precursors for novel drug development [3, 4].

The antimicrobial activity of medicinal plants refers to their ability to inhibit or kill pathogenic microorganisms [5]. The use of plant extracts with antimicrobial properties dates back at least 2000 years, with historical records from ancient Greek and Egyptian civilizations documenting their application in treating infections [6]. Many traditional medicine systems worldwide rely on plant-derived antimicrobial agents, and modern research has further validated their efficacy, leading to the development of new drugs [7]. However, the widespread use of synthetic antibiotics has led to the emergence of drug-resistant microorganisms, which can adapt to changing environments over time [8]. Additionally, many conventional antibiotics have harmful side effects, necessitating the search for alternative natural sources. Higher plants, rich in antimicrobial compounds, present a promising solution [9, 10]. Before the advent of modern medicine, medicinal plants were the primary means of treating diseases. Ancient civilizations revered these plants, believing in nature's inherent healing power. According to the World Health Organization (WHO), approximately 80% of the population in developing countries still relies on plant-based medicine, with 3.3 billion people incorporating medicinal plants into their daily healthcare practices [11].

The Scrophulariaceae family belongs to the Asterids, a large clade of dicotyledonous plants that represents one of the most diverse groups of angiosperms. Asterids include several economically important families, such as those of carrots, potatoes, tea, and tomatoes. Key characteristics of this group include the presence of iridoids, unitegmic and tenuinucellate ovules, and sympetalous corollas [12].

Scrophulariaceae is classified under the order Lamiales, which comprises about 24 families. Due to taxonomic revisions, many former members of Scrophulariaceae have been reclassified into Plantaginaceae, leaving it with a limited number of genera [13]. The name Scrophularia is derived from the Latin scrofula, referring to the plant's historical use in treating glandular swellings. The family includes approximately 56 genera and 1700–1800 species, with around 30 genera formally identified. These plants are distributed across both temperate and tropical regions, with 30% being annual and 40% perennial [14,15].

The flowers of Scrophulariaceae exhibit zygomorphic (bilateral) symmetry, though some species display actinomorphic (radial) traits. They

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are typically hermaphroditic and grow in leaf axils. The sepals and petals are fused (connate), and the stamens—usually in pairs of 2 or 4—are attached to the petals. A fifth, often non-functional staminode may also be present. The gynoecium is syncarpous, consisting of two fused carpels with a superior, bilocular ovary containing numerous axile ovules. The fruit is a two-valved capsule bearing tiny seeds. Leaves lack stipules and are arranged oppositely or in whorls [16,17].

Vegetative morphology varies widely within the family. While Paulownia species are trees, Halleria includes shrubs, and Wightia features woody climbers. Some genera are geophytic, but the most common growth forms are annual and perennial herbs. A prominent taproot is typical, though in some species, adventitious roots become dominant if the primary root dies during germination. Haustoria—specialized structures for nutrient absorption—are present in parasitic members. Secondary haustoria arise from lateral or adventitious roots, while primary haustoria develop from swellings on the main root [18]. Certain members of the subfamily Rhinanthoideae are hemiparasitic, attaching to the roots of other flowering plants to obtain water and nutrients while retaining photosynthetic ability. Stem morphology also varies; for instance, Limosella has condensed internodes, whereas Cymbalaria exhibits creeping stems [18].

Scrophulariaceae species are distributed globally and hold significant economic value as timber, ornamental plants, and medicinal resources [19]. The family includes both autotrophic and parasitic herbs and shrubs. Parasitic members, despite possessing chlorophyll, rely on host plants for sustenance and typically have fleshy leaves, whereas autotrophic species exhibit non-succulent foliage [20]. In this study, the antimicrobial activities of plants from the genus Scrophularia (family Scrophulariaceae) were evaluated

MATERIAL AND METHODS

Preparation of Plant Material

Extraction Solvent

To optimize the extraction of antimicrobial compounds from the aerial parts of the plants, aqueous and ethanol solvents were used in this study [21]. The aerial parts were crushed using a mortar. Fifty grams of the plant material was mixed with aqueous (40%) and ethanolic (60%) solutions to prepare the extracts. The mixture was placed in a 300 mL flask and agitated on a rotary shaker for three days. Afterward, the extract was filtered using Whatman No. 1 filter paper. The filtrates were collected in a volumetric flask. To concentrate the extracts, the ethanol was evaporated under reduced pressure using a rotary evaporator at 40°C. The dried extracts were then stored in sterile glass vials until further use. For stock solution preparation, 1 gram of the plant powder was dissolved in 10 mL of ethanol. Additionally, separate aliquots were placed in pre-weighed freeze-dryer bottles and lyophilized for 24 hours until completely dry.

Test Microorganism

The antimicrobial activity of the extracts was assessed against 3 bacterial strains and one strain of fungi, which were all obtained from the biology laboratory.

Disk Diffusion

Antibacterial and antifungal activities were determined using the disc diffusion test method [22]. Plant extracts were tested against fourteen bacteria and one fungal species. In this method, the bacterial inoculum was adjusted to 4 different concentrations, which were 10 ug/dis, 50 ug/dis, 100 ug/dis, and zero. The Microorganisms were inoculated onto the entire surface of a Mueller-Hinton agar (MHA) plate with a sterile cotton-tipped swab to form an even lawn.

The disk diffusion method allows for the simultaneous testing of a large number of antimicrobials in a relatively easy and flexible manner. The paper disks (6 mm in diameter; BD Diagnostic Systems) impregnated with diluted plant extract solution (10, 50, 100 µl) were placed on the surface of each MHA plate using a sterile pair of forceps. The bacterial plates were incubated at 37±0.1 °C for 24 h, while fungal plates were incubated at 28±0.1 °C for 48 h. The diameter of the inhibition zone was measured after 24 h incubation using a ruler or caliper [23].

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of an antimicrobial agent is the lowest concentration of the agent inhibits growth. We determined the concentration in the laboratory by incubating a known quantity of bacteria with s The extracts were added into a Mueller Hinton agar that was prepared from a hydrated base. The PH of the agar must be between 7.2 and 7.4 at room temperature, with each plate containing a different concentration of extract. Within 15 minutes of adjusting the inoculum to the 0.5 McFarland turbidity standards [24]. The suspension was mixed and diluted to obtain the final concentration of 5x10 CFU/ ml. The 2, 0 ml of the original suspension was delivered to the 38 ml of water dilution. The inoculator was transferred 0,01 ml (1:10 dilution) into each well. The MIC panel was inoculated with care to avoid splashing from one well to another. After an incubation of 18-24 hours at 37 °C, the end point of MIC was noted as it represents the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye [25].

Statistical analysis

For statistical analysis, descriptive statistics and tests for homogeneity of variance were used to assess differences in the inhibitory zone sizes produced by the extracts against different microorganisms.

RESULTS

The antimicrobial effects of *Scrophulariaceae* extract were studied for their activity against microorganisms, *Bacillus subtilis, Candida albicans, Enterococcus faecalis, and Staphylococcus epidermidis.* It was seen that the *scrophlariaceae* had shown an effect against *Bacillus subtilis, Staphylococcus epidermidis.* But the extract of this plant did not have *an* effect against *Candida albicans or Enterococcus faecalis,* as shown in Table 1.

Table 1. Antimicrobial activity of Scrophulariaceae by the disk diffusion method.

Microorganisms	*Scrophulariaceae* µ/disc		
	10	50	100
Baciluss subtilis	X	6	11
C. albicans	X	X	X
E. faecalis	X	X	X
S. epidermidis	X	X	8

The antimicrobial activity of Scrophulariaceae plant extracts against various microorganisms (bacteria and fungi) is detailed below, with data presented in both tabular and graphical formats. The graph illustrates the effects of three extract concentrations (10 µL, 50 µL, and 100 µL) on different microbial strains, with each concentration represented by a distinct color: green (10 µL), red (50 µL), and blue (100 µL).

The extracts exhibited activity against Bacillus subtilis and Staphylococcus epidermidis, as tested at two concentrations (50 µL and 100 µL; see Table 1). The largest inhibition zones were observed at the 100 µL concentration: 11 mm for B. subtilis and 8 mm for S. epidermidis (Figure 1). However, the extracts showed no significant effect against the other tested bacteria and fungi.

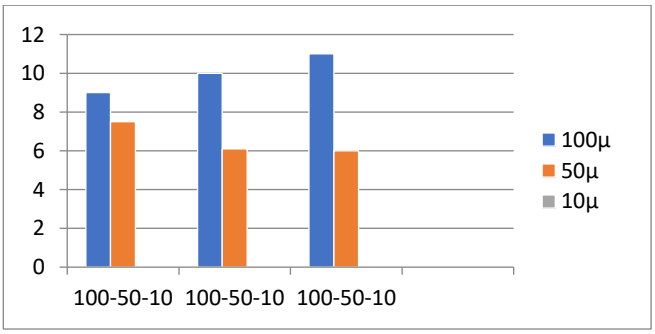

Figure 1. Antimicrobial effect zone diameter of three concentrations of scrophulariaceae extract against B. subtilis.

Minimum Inhibitory Concentration (MIC)

The result in the minimal inhibitory concentration (MIC) method of scrophlariaceae extract was observed as 100 µg/ml against *S. epidermidis, B. Subtillis* (100 µg/ml) against *S. epidermidis,* and a lower inhibitory concentration (50 µg/ml) against *B. subtillis.* The lowest MIC result observed against was while no effect with C. *albicans and E. faecalis* was shown in Table 2.

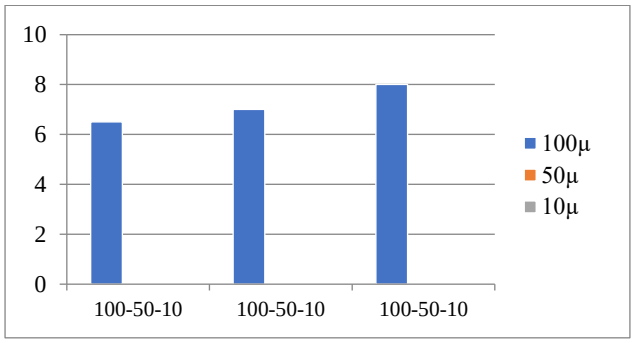

Figure 2. Antimicrobial effect zone diameter of three concentrations of Scrophulariaceae extract against S. epidermidis.

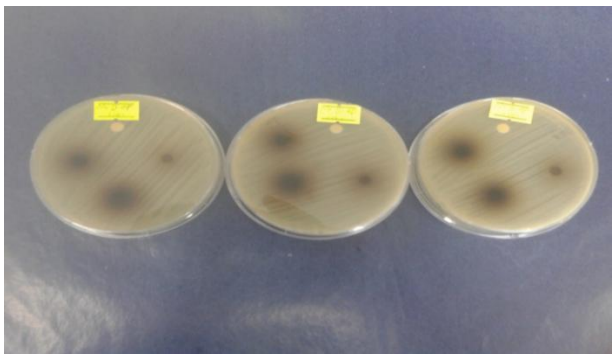

Figure 3. Antimicrobial zone of scrophlariaceae against B subtilis

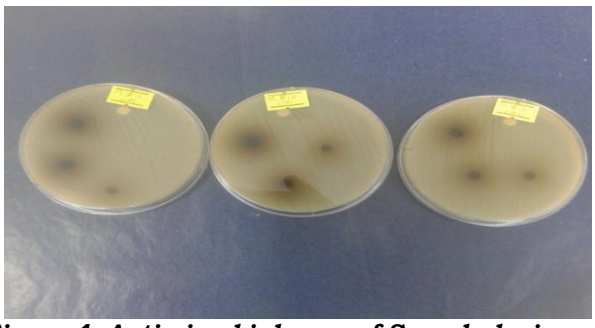

Figure 4. Antimicrobial zone of Scrophulariaceae against S. epidermidis

Table 2. Minimum inhibitory concentration results.

Extract	*C. albicans*	*B. Subtillis*	*S. epidermidis*	*E. faecalis*
scrophlariaceae	X	50	100	X

DISCUSSION

The extracts collected from different species of Scrophulariaceae exhibited varying antimicrobial activity against Bacillus subtilis, Candida albicans, Enterococcus faecalis, and Staphylococcus epidermidis. It was observed that Scrophulariaceae extracts showed antimicrobial effects against B. subtilis, S. epidermidis, E. faecalis, and C. albicans, while no activity was detected against some other microorganisms. The differences in antimicrobial activity were attributed to variations in extract concentration and potency.

Şener & Dülger [26] investigated the antimicrobial activity of ethanol extracts from Scrophulariaceae leaves against six different microorganisms using

the disk diffusion and microdilution methods. They reported strong antimicrobial effects against E. faecalis, Proteus mirabilis, and C. albicans, with inhibition zones of 20 mm, 18 mm, and 20 mm, respectively. In our study, V. domulosum exhibited activity against C. albicans (15 mm inhibition zone), while Scrophulariaceae showed activity against E. faecalis (8 mm inhibition zone). The discrepancies in results may be due to differences in the Scrophulariaceae species used.

Batool & Abu-Hadi [27] studied the antibacterial and antifungal properties of Scrophulariaceae against C. albicans, B. subtilis, Staphylococcus aureus, S. epidermidis, Escherichia coli, and Pseudomonas aeruginosa. Using the well diffusion method, they observed antimicrobial activity against all tested microorganisms except P. aeruginosa and C. albicans, whereas the serial dilution method showed activity against all. In our study, V. speciosum displayed activity against B. subtilis (8 mm inhibition zone at 50 µg/mL and 100 µg/mL) and C. albicans (10 mm inhibition zone at 100 µg/mL), but no activity was detected against P. aeruginosa. These differences may stem from the use of ethanol extracts and the disk diffusion method in our experiments.

CONCLUSION

The findings of this study demonstrate that extracts from various Scrophulariaceae species exhibit selective antimicrobial activity against Bacillus subtilis, Candida albicans, Enterococcus faecalis, and Staphylococcus epidermidis, while showing no effect against certain other microorganisms. The variations in antimicrobial efficacy can be attributed to differences in extract concentration, potency, and the specific species of Scrophulariaceae used. Despite these variations, the consistent antimicrobial effects against key pathogens highlight the potential of Scrophulariaceae extracts as a source of bioactive compounds for antimicrobial applications. Further research is needed to standardize extraction methods, identify active constituents, and evaluate their mechanisms of action for potential therapeutic use.

Conflict of interest. Nil

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