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In Vitro Analysis of Antioxidant and Antifungal Potential of *Swertia Chirata*

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ABSTRACT

The global occurrence of opportunistic fungal infections affecting both humans and animals is steadily increasing. *Swertia chirata* is an ancient herb from the Gentianaceae family, renowned for its medicinal significance. In present study, *S. chirata* samples collected from forests of Murree, Pakistan and were evaluated for antifungal and antioxidant activity. Bioactive compounds were extracted using ethanol and distilled water through the soxhlet extraction method. The antifungal properties of both aqueous and ethanolic extracts of *S. chirata* were evaluated against *Fusarium oxysporum* using the disc diffusion technique. Furthermore, the antioxidant capacity of the extracts was assessed through the phosphomolybdenum assay. Higher concentrations of the plant extract resulted in greater zones of inhibition. No zone, 7±0.53 mm, 11±0.33 mm and 13±0.18 mm zones of inhibitions were observed at 0.01 g, 0.02 g, 0.04 g and 0.08 g in 10 ml of ethanolic extracts respectively. For 0.01g and 0.02g contraction of *S. chirata* in 10 ml aqueous extracts, no zones of inhibition were observed, however, 8±0.29 mm and 11±0.41 mm zones were observed for 0.04 g and 0.08 g extracts respectively. Total antioxidant capacity of *S. chirata* elevated by increasing the concentration of both aqueous and ethanolic extracts. *S. chirata* extract is recommended for *in-vivo* antifungal and antioxidant activity testing.

INTRODUCTION

Estimates of fungal disease incidence and mortality are imprecise. Infectious diseases rank as the second most common cause of death worldwide, after cardiovascular diseases. Although the majority of infections are attributed to viruses and bacteria, there is a growing global incidence of opportunistic fungal infections impacting humans and animals (Gnat et al., 2021). This issue is receiving increasing attention, particularly due to the rising incidence of persistent and recurrent fungal infections (mycoses). Traditional medicine remains a significant component in fulfilling the primary healthcare needs of populations across different parts of the world (Aschale et al., 2021). Medicinal plants are abundant in phytochemicals that can be structurally modified and developed into novel pharmaceutical drugs (Ugboko et al., 2021).

Various studies have confirmed that plant extracts possess antibacterial activity against a wide range of bacterial and fungal species (Egamberdieva, et al., 2021). Several plants possess essential oils and proteins exhibit significant antibacterial and antifungal activities (Sameen et al., 2025). Compared to synthetic antibiotics, plant-derived antimicrobial agents offer several benefits, including less toxicity, few or no side effects and consists of wide variety of bioactive compounds. These compounds can disrupt multiple mechanisms of fungal growth, thereby reducing the chances of survival (Shereen et al., 2024). Identifying potential drugs from plant sources is essential for managing fungal infections. Furthermore, documenting the most commonly used plant components and their routes of administration is crucial for guiding future drug formulation research (Aschale et al., 2021).

Swertia chirata, widely recognized as chiryatah, is a medicinal herb that belongs to the Gentianaceae family. It is native to the temperate zones of the Himalayan region and the Khasi Hills located in Meghalaya. It typically grows at elevations ranging from 1100 to 3000 meters (Verma et al., 2024). Traditionally, it has been utilized to manage a range of health conditions such as liver and digestive

disorders, heart ailments, malaria, scorpion bites, skin diseases, and diabetes. The herb is renowned for its wide array of pharmacological effects (Swati et al., 2023). Additionally, it holds a prominent place in traditional healthcare systems including as ayurveda and other conventional healing practices (Mazumder et al., 2023). Therefore, current study aims to evaluate the antifungal activity of *S. chirata* extracts using different solvents against fungal pathogen. The results of present investigation may provide valuable insights into the potential use of plant-derived therapeutics for the treatment of mycosis.

MATERIALS AND METHODS

Plant sample collection

Swertia chirata was first identified and collected from the forests of Murree in Punjab, Pakistan. The current study was conducted in the Chemistry Laboratory, Department of Chemistry, University of Sialkot.

Sample Processing

Firstly, leaves and stems were separated and carefully cleansed with sterile distilled water to remove any impurities or contaminants. Following the cleaning process, the plant materials were dried in a hot air oven at 60 °C for four hours (Zambra et al., 2021). Once dried, they were finely ground into powder and stored in a sterile bottle for further investigation.

Preparation of extract

The powdered stem and leaf samples were extracted for bioactive compounds using a Soxhlet apparatus, using ethanol and distilled water as solvents. The extraction process was carried out at 60 °C over a period of 48 hours. Following extraction, the obtained solutions were allowed to cool to room temperature and subsequently passed through Whatman No.1 filter paper for filtration. The resulting filtrates were then evaporated to complete dryness using a flash evaporator. The completely dried extracts were transferred into clean, sterilized containers for subsequent use (Abubakar et al., 2020).

Test fungal strain

The fungal strain (*Fusarium oxysporum*) used in this investigation was obtained from the Department of Microbiology, University of Sialkot.

Antifungal Activity Assay

Antifungal activity was determined by agar well diffusion method following Laxmi et al. (2011). Fungal cultures were refreshed by inoculating into broth media and incubated at 27°C for 72 hours. Potato Dextrose Agar (PDA) plates were prepared. Each plate was inoculated with 0.1 mL of a fungal spore suspension (*Fusarium oxysporum*), which was evenly spread across the surface of the agar.

After allowing the suspension to adsorb for 20 minutes, wells were carefully made in the agar. Test samples at different concentrations were subsequently introduced into each well. Positive and negative controls were also employed using nystatin (as standard antifungal agent) and 10% Tween 80, respectively. The plates were incubated at 27 °C for 72 hours, followed by measurement of the inhibition zone diameters surrounding each well to evaluate antifungal activity

Total Antioxidant Capacity

Total antioxidant capacity of extracts was determined following the procedure outlined by Prieto et al. (2010). In this assay, 0.2 mL of the plant ethanolic and aqueous extracts of different concentrations were added in 4 mL of a reagent mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. For the blank, 0.2 mL of ethanol was added in 4 mL of the same reagent solution. The reaction mixture was incubated under appropriate conditions, and the absorbance of the test sample was recorded at 695 nm using a spectrophotometer.

RESULTS

Assessment of Antifungal Activity

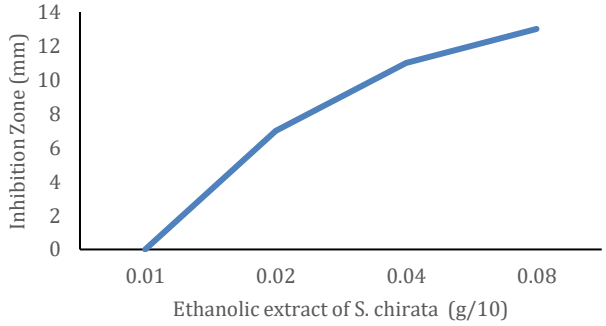
The antifungal activity *S. chirata* extracts prepared with distilled water and ethanol was evaluated using the well diffusion method. The activity was assessed by measuring the zones of inhibition formed at different concentrations of the extracts. Both ethanol and aqueous extracts of *S. chirata* exhibited antifungal effects, producing clear inhibition zones. An increase in the concentration of the plant extracts corresponded to an increase in the diameter of the inhibition zones.

Antifungal Activity of Ethanol Extracts

In the case of the ethanolic extracts, the first concentration (0.01 g/10 mL) showed no antifungal activity against *Fusarium oxysporum*. The second concentration (0.02 g/10 mL) exhibited antifungal activity, producing an inhibitory zone of 7 mm. The third concentration (0.04 g/10 mL) demonstrated increased antifungal efficacy, with an inhibition zone of 11 mm. The fourth and highest concentration (0.08 g/10 mL) showed the strongest antifungal activity, resulting in a 13 mm zone of inhibition (Fig 1).

Figure 1

Antifungal Activity of Ethanolic Extracts of S. Chirata (g/10) against Fusarium Oxysporum.



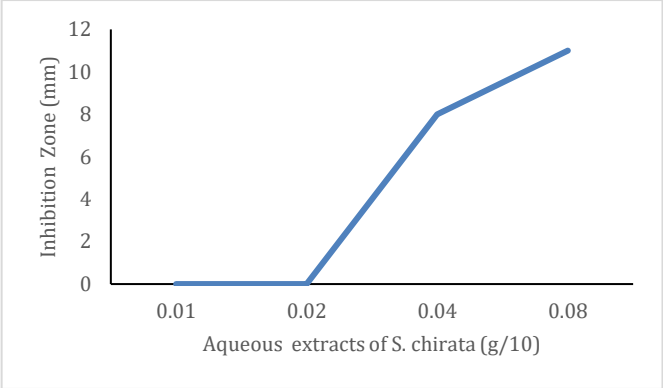
Antifungal activity of aqueous extracts

In the case of the distilled water extracts of *S. chirata*, the first two extract solutions (0.01 g/10 mL and 0.02 g/10 mL) exhibited no antifungal activity against *F. oxysporum*, as no zone of inhibition was observed. However, the third extract (0.04 g/10 mL) showed noticeable antifungal activity, producing a zone of inhibition measuring 8 mm. Similarly, the fourth extract (0.08 g/ 10 mL) also demonstrated antifungal activity, with a zone of inhibition

measuring 11 mm (Fig. 2).

Figure 2

Antifungal Activity of Aqueous Extracts of S. chirata (g/10) against F. Oxysporum.



Evaluation of total antioxidant potential of *S. chirata*

Absorbance of both aqueous and ethanolic extracts was taken at 695 nm to determine the total antioxidant capacity of *S. chirata*. Increase in absorbance is correlated with increased total antioxidant potential.

Total antioxidant potential of ethanolic extract of *S. chirata*

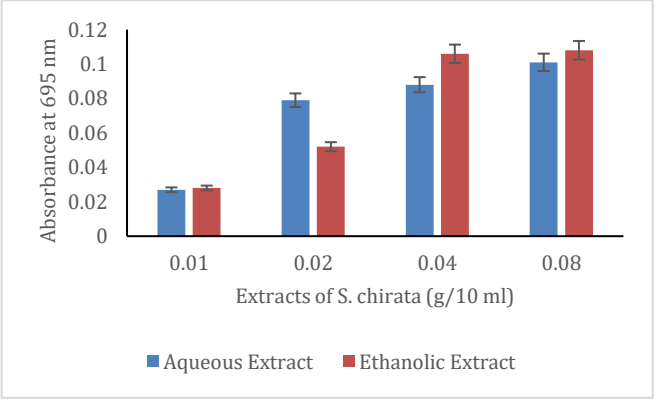
In the case of the ethanolic extracts, the first sample, with a concentration of 0.01 g/10 mL, recorded an absorbance of 0.028. The second extract, at a concentration of 0.02 g/10 mL, showed a higher absorbance value of 0.052. The third extract, containing 0.04 g/10 mL, exhibited a further increase in absorbance, reaching 0.106. Finally, the fourth extract, which had the highest concentration of 0.08 g/10 mL, displayed the highest absorbance, measuring 0.108 (Fig. 3).

Total antioxidant capacity of aqueous extract of *S. chirata*

For the aqueous extracts, the first sample with a concentration of 0.01 g/10 mL showed an absorbance of 0.027. The second extract, at a concentration of 0.02 g/10 mL, exhibited a higher absorbance of 0.079. The third extract, with a concentration of 0.04 g/10 mL, demonstrated a further increase in absorbance to 0.088. Lastly, the fourth and most concentrated extract (0.08 g/10 mL) recorded the highest absorbance value of 0.101 (Fig 3).

Figure 3

Total Antioxidant Capacity of Aqueous and Ethanolic Extracts of S. Chirata (g/10).



DISCUSSION

Swertia chirata holds significant value in both traditional and folk medicine for the treatment of various ailments. The plant contains a range of bioactive secondary metabolites, which serve as valuable sources for developing medicines aimed at combating numerous microbial infections and enhancing overall human health (Dey et al., 2020; Verma et al., 2024). In the present study, the antifungal and antioxidant properties of *S. chirata* collected from the Murree Forest in Punjab were evaluated using ethanol and distilled water extracts.

Human fungal infections have long been an overlooked area in disease research, despite being responsible for over 1.5 million deaths annually. However, over the past decade, significant progress has been made in understanding the pathophysiology of these infections, with major advances in identifying both host and pathogen factors that influence the manifestation and severity of the diseases (Brown et al., 2024). This study examined the antifungal potential of *S. chirata* plant extracts against the human pathogenic fungus *Fusarium oxysporum*. This pathogen is known to cause infections not only in humans but also in a wide range of plants, including essential staple crops and ornamental species, and it plays a significant role in food spoilage (Ayhan et al., 2025; Cighir et al., 2023; Thapa et al., 2022; Qi et al., 2022). In current study, the zone of fungal growth inhibition increased progressively with higher concentrations of both ethanolic and aqueous extracts. The findings indicate that *S. chirata* plant extracts possess significant antifungal activity against *F. oxysporum.*

For the ethanolic extracts, the lowest concentration (0.01 g/10 mL) did not exhibit any antifungal activity against *F. oxysporum*. At the next concentration level (0.02 g/10 mL), antifungal activity was observed, with an inhibition zone measuring 7 mm. Increasing the concentration to 0.04 g/10 mL resulted in enhanced antifungal efficacy, as evidenced by an 11 mm zone of inhibition. The highest concentration tested (0.08 g/10 mL) produced the most significant antifungal effect, yielding a 13 mm inhibition zone.

For the distilled water extracts of *S. chirata*, the first two extract having concentrations of 0.01 g/10 mL and 0.02 g/10 mL showed no antifungal activity against *F. oxysporum*, as indicated by the absence of any inhibition zone. In contrast, the third concentration (0.04 g/10 mL) exhibited clear antifungal activity, resulting in an inhibition zone measuring 8 mm. The highest concentration tested (0.08 g/10 mL) also demonstrated antifungal effectiveness, producing an inhibition zone of 11 mm. Similar antibacterial properties have been documented in previous studies (Shereen et al., 2024; Khan et al., 2018; Roy et al., 2015). To the best of our knowledge, this is the first report demonstrating the antifungal activity of *S. chirata*.

Antioxidants are biologically active compounds, primarily found in fruits, vegetables, grains, and herbs, that help protect the body from damage caused by reactive oxygen species (ROS). These naturally occurring phytochemicals serve a vital role in defending cells against oxidative stress by neutralizing free radicals and other harmful oxidizing agents (Kazmi et al., 2019). Cells have developed multiple

defense mechanisms that rely on both water-soluble and fat-soluble antioxidants, as well as antioxidant enzymes. Many of these protective systems in the human body are largely dependent on nutrients obtained through the diet. Plant-derived antioxidants are biologically active compounds that safeguard the body against damage resulting from oxidative stress caused by free radicals (Tumilaar et al. 2024).

In present study, the total antioxidant capacity of *S. chirata* was assessed by measuring the absorbance of both its aqueous and ethanolic extracts at 695 nm. A higher absorbance value indicates a greater total antioxidant potential. The results of the total antioxidant capacity assay for the ethanolic extracts of *S. chirata* demonstrated a clear concentration-dependent increase in absorbance values, indicating enhanced antioxidant potential with increasing extract concentration. At the lowest concentration (0.01 g/10 mL), the absorbance was recorded at 0.028, suggesting minimal antioxidant activity. As the concentration doubled to 0.02 g/10 mL, the absorbance value increased to 0.052, reflecting a corresponding rise in antioxidant capacity. Further increase in concentration to 0.04 g/10 mL resulted in a more pronounced absorbance of 0.106, highlighting a significant improvement in the extract's ability to reduce molybdenum (VI) to molybdenum (V), which is indicative of stronger antioxidant properties (Untea et al., 2018). Interestingly, the highest concentration tested (0.08 g/10 mL) produced an absorbance of 0.108, only marginally higher than the previous concentration. This slight increase suggests a potential plateau effect, where further increases in extract concentration may not proportionally enhance antioxidant capacity.

The antioxidant activity of the aqueous extracts of *Swertia chirata* also showed a concentration-dependent trend, similar to the ethanolic extracts. The extract with the lowest concentration (0.01 g/10 mL) exhibited an absorbance of 0.027, indicating a relatively low level of antioxidant activity. As the concentration increased to 0.02 g/10 mL, there was a notable rise in absorbance to 0.079, suggesting a significant enhancement in antioxidant potential. At a concentration of 0.04 g/10 mL, the absorbance further increased to 0.088, reinforcing the positive correlation between extract concentration and antioxidant activity. The highest concentration tested (0.08 g/10 mL) resulted in the highest absorbance value of 0.101, confirming that the antioxidant potential continued to improve with increasing concentration.

These findings suggest that the both ethanolic and aqueous extracts of *S. chirata* exhibits a strong dose-dependent antioxidant response up to a certain concentration, beyond which the increase in activity becomes less pronounced. This trend may be attributed to the saturation of active antioxidant compounds. This plateau effect may result from the saturation of antioxidant-active compounds in the assay system. Such trends are consistent with previous research highlighting plant-based extracts typically yield greater antioxidant activity due to the cumulative presence of bioactive compounds, particularly phenolics and flavonoids (Dai & Mumper, 2015; Shahidi & Ambigaipalan, 2015). Our findings align with earlier research and further validate the antioxidant potential of *S. chirata* (Shereen et al., 2024; Verma et al., 2024; Deepak et al., 2021; Yadav et al., 2024).

In summary, the results of this study indicate that *S. chirata* extracts possess promising antifungal activity against pathogenic fungi. Nonetheless, additional research is required to explore the underlying mechanisms of action, as well as to evaluate the safety and toxicity profiles of both the extracts.

CONCLUSION

This study demonstrates that *S. chirata*, a medicinal herb from the Gentianaceae family, possesses notable antifungal and antioxidant properties. Extracts obtained from samples collected in the Murree forests of Pakistan showed concentration-dependent biological activity. The ethanolic extracts exhibited significant antifungal effects against *F. oxysporum*, with increasing zones of inhibition observed at higher concentrations. Aqueous extracts also showed antifungal activity, though only at higher concentrations. Furthermore, both aqueous and ethanolic extracts displayed enhanced total antioxidant capacity with increasing concentration, as determined by the phosphomolybdenum assay. These findings suggest that *S. chirata* contains bioactive compounds with potential therapeutic applications. However, further *in vivo* studies and detailed toxicity assessments are essential to validate its efficacy and safety for clinical or pharmaceutical use.

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