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Molecular Docking and Antileishmanial Potential of Isolated Compounds from *Asparagus gracilis*

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ABSTRACT: **Leishmaniasis** remains a neglected tropical disease with high global disease burden and limited safe therapeutic options. Medicinal plants offer an important source of bioactive compounds for drug development. This study aimed to isolate and characterize bioactive compounds from *Asparagus gracilis* and evaluate their antileishmanial potential using both *in vitro* and *in silico* approaches. The plant was shade-dried, extracted with methanol, and fractionated using solvents of increasing polarity. Bioactive fractions were subjected to column chromatography, and compounds were characterized by proton nuclear magnetic resonance (^1H-NMR). Antileishmanial activity was assessed against *Leishmania tropica* promastigotes using the MTT assay. Molecular docking was performed against leishmanolysin GP63 (PDB: 1LML) using MOE software. The ethyl acetate fraction displayed the highest antileishmanial activity with an IC_{50} value of 13.5 µg/mL. Ferulic acid was isolated at 4% ethyl acetate–n-hexane solvent system and identified as the major bioactive compound. The compound exhibited dose-dependent inhibition of *L. tropica* promastigotes with an IC_{50} of 11.1 µg/mL. Docking studies revealed strong binding affinity with GP63, showing four hydrogen bonds and a binding energy of −5.9 kcal/mol. In comparison with the standard antileishmanial drug Glucantime (IC_{50} = 6.9 µg/mL), the isolated compound demonstrated statistically significant inhibitory potential ($p < 0.05$), highlighting its promising biological activity. The results suggest that ferulic acid from *A. gracilis* exhibits promising antileishmanial activity. This compound can serve as a potential lead for the development of new antileishmanial agents.

Keywords: *Asparagus gracilis*; Ferulic acid; Leishmaniasis; Molecular docking; Antileishmanial activity

1. INTRODUCTION

Medicinal plants have played a central role in health care since ancient times. Approximately 80% of populations in developing countries rely on herbal remedies for primary health care needs (Govind, 2011; Ozkan et al., 2016; Sofowora et al., 2013). A significant number of modern pharmaceuticals are derived from plant-based compounds, reflecting their therapeutic potential (Abelson, 1990; Fowler and Agriculture, 2006; Wink, 2015). Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus *Leishmania*, transmitted through the bite of infected sandflies Globally, an estimated 12 million people are infected, and two million new cases are reported annually Pakistan faces a particularly high prevalence of cutaneous leishmaniasis, especially in rural regions

(Desjeux and diseases, 2004; Murray et al., 2005; Torres-Guerrero et al., 2017).

Current treatment options are limited, costly, and associated with adverse effects and drug resistance (Lindoso et al., 2012; Monzote, 2009; Pradhan et al., 2022). Resistance to pentavalent antimonials, amphotericin B, and miltefosine has been increasingly reported, leading to therapeutic failure in endemic regions. This highlights the urgent need for novel, safe, and cost-effective antileishmanial agents.

Asparagus gracilis, locally known as Shaghandal, belongs to the family Asparagaceae and has been traditionally used to treat various ailments including skin infections, inflammatory conditions, and diabetes(Hamdi, 2016; Shah et al., 2014). Members of the *Asparagus* genus are known to contain diverse phytochemicals such as flavonoids, alkaloids, saponins, terpenoids, and glycosides with reported antimicrobial and antiparasitic properties This study aimed to isolate active compounds from *A. gracilis* and assess their antileishmanial activity through both *in vitro* and molecular docking approaches.

2. MATERIALS AND METHODS

2.1. Plant collection and extraction

A. gracilis was collected from Islamabad, Pakistan during the flowering season in March 2023. Botanical authentication was carried out at the Department of Botany, and a voucher specimen was deposited. The plant material was washed, shade-dried, and powdered. Methanolic extraction was performed using maceration (Hussain et al., 2019), seven days with occasional stirring and the extract was concentrated under reduced pressure at 40 °C using a rotary evaporator.

2.2. Fractionation and isolation

The crude extract was fractionated sequentially with n-hexane, chloroform, ethyl acetate, and water. Fractions were evaporated to dryness and stored at 4 °C. The ethyl acetate fraction, showing the highest activity, was subjected to column chromatography using ethyl acetate–n-hexane solvent system (0–9% gradient). Pure compound T1 was isolated at 4% ethyl acetate–n-hexane. Column chromatography was performed on a 70 × 2.5 cm glass column packed with silica gel (70–230 mesh, 200 g). Approximately 3 g of ethyl acetate fraction was loaded using a solvent gradient of n-hexane:ethyl acetate (0–9%) at a flow rate of 1 mL/min. Fractions (25 mL each) were collected and monitored by TLC (silica gel 60 F254, visualized under UV 254/366 nm using methanol:chloroform 3:7). Similar fractions were pooled and concentrated. Fraction F1 (yield: 65 mg, purity 95% by TLC) afforded a pure compound designated as T1.

2.3. Characterization of isolated compound

Isolation of the Compound

Column chromatography was performed using a 70 cm glass column packed with silica gel (70–230 mesh) as the stationary phase. The ethyl acetate fraction was pre-adsorbed onto a small amount of silica gel to form a slurry, which was air-dried and carefully loaded into the column to prevent cracking. Elution was carried out using a gradient of n-hexane and ethyl acetate, starting from pure n-hexane and gradually increasing polarity up to 9% ethyl acetate. Six fractions (F1–F6) were collected. Re-chromatography of fraction F1 with 4% ethyl acetate–n-hexane yielded a pure compound, designated as T1.

2.4. *In vitro* antileishmanial assay

The activity of plant fractions and ferulic acid was tested against *L. tropica* promastigotes using the MTT assay (Shah et al., 2019a). Different concentrations (31.25–500 µg/mL) were incubated for 72 h at 24 °C. Glucantime served as a positive control. Absorbance was measured at 540 nm using a microplate reader, and IC_{50} values were calculated. Promastigotes (passage 3–5) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 µg/mL streptomycin at pH 7.2. Cultures were maintained at 24 ± 1 °C without CO_2. For each assay, 1×10^6 parasites/mL were inoculated into 96-well plates.

2.5. Molecular docking

Docking was performed using MOE 2016 against leishmanolysin GP63 (PDB: 1LML). The docking scores and hydrogen bond interactions were analyzed using Biovia Discovery Studio Visualizer (Shah et al., 2019b).

2.6. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± SD. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$ considered significant).

3. RESULTS

3.1. Phytochemical Screening

The preliminary phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins, glycosides, tannins, terpenoids, and phenolic compounds in the methanolic extract (Table 1).

3.2. Characterization of isolated compound

The isolated compound was characterized using thin-layer chromatography (TLC), ^{1}H-NMR and ^{13}C NMR spectroscopy. The structure was confirmed as ferulic acid (4-hydroxy-3-

methoxycinnamic acid) by comparing spectral data with reported literature (Figure 1).

Table 1. Phytochemical screening result of crude extract

Phytochemical Class	Observations
Alkaloid	+
Carbohydrates	+
Flavonoid	+
Glycosides	+
Phenol	+
Saponin	+
Sterols	+
Tannin	+
Terpenoids	+

+ Sign indicate the presence of Phytochemical class

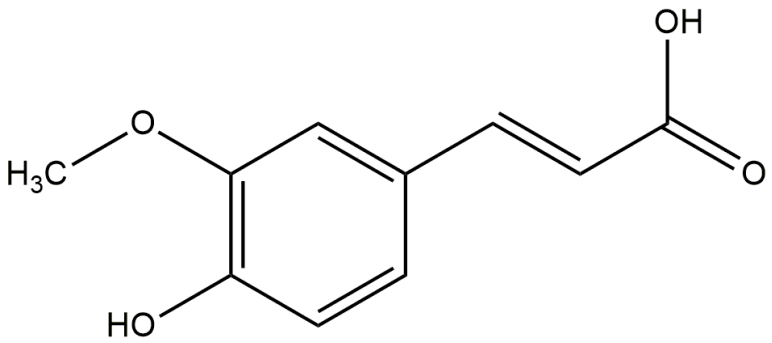

Figure 1. Structure of ferulic acid (4-hydroxy-3-methoxycinnamic acid)

3.3. Antileishmanial activity

The ethyl acetate fraction exhibited the highest inhibition of promastigote growth, with an IC_{50} value of 13.5 µg/mL. Ferulic acid showed dose-dependent inhibition with an IC_{50} of 11.1 µg/mL. The standard drug Glucantime had an IC_{50} of 6.9 µg/mL (Table 2). The difference between ferulic acid and Glucantime was statistically significant ($p < 0.05$), confirming the moderate yet meaningful inhibitory potential of the natural compound.

Table 2: Antileishmanial activity of plant extract, isolated compound, and standard drug.

Fraction/Compound	Concentration (µg/ml)	Inhibition (%)	IC_{50} (µg/ml)
Ethyl-Acetate fraction extract of *Asparagus gracilis*	500	94	9.9
	250	77.6	
	125	28.6	
	62.5	9.2	

	31.25	----	
4-hydroxy-3-methoxycinnamic acid (Compound isolated)	500	60.4	11.1
	250	33.5	
	125	12.6	
	62.5	1.6	
	31.25	---	
Glucantime (Standard)	500	97	6.90
	250	82.2	
	125	62.5	
	62.5	17.09	
	31.25	----	

3.4. Molecular docking analysis

Ferulic acid demonstrated a binding energy of −5.9 kcal/mol (table 3) with GP63, forming four hydrogen bonds with residues SER-234, GLU-166, THR-129, and THR-130. Van der Waals interactions were observed with TRP-61, ALA-128, and PHE-177 (Figure 2).

Table 3. Bonding energy of Ferrulic acid

Ligand	Target proteins	E-value (Kcal/mol)	No of H bonds	Binding residues forming H bonds
Compound	1LML	-5.9	4	SER-234

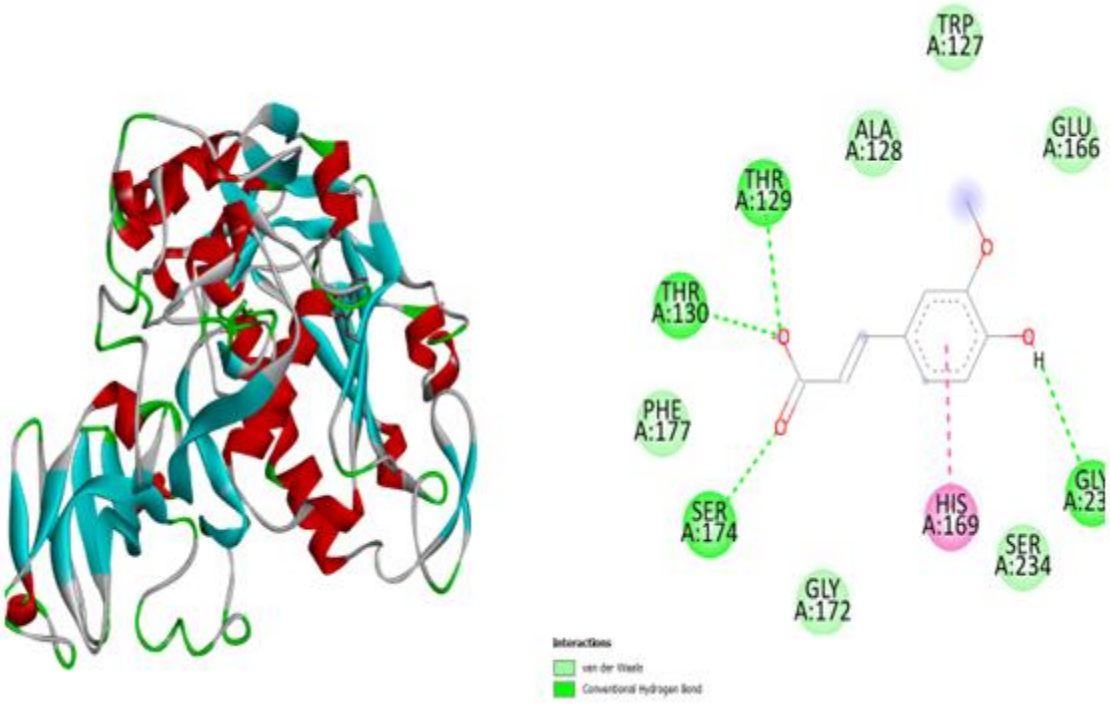

Figure 2: In silico docking of Ferulic acid with gp63 (PDB code: 1LML) A. 3D interaction of complex B. 2D interaction of complex.

4. Discussion

Medicinal plants have been an important source of novel therapeutic molecules (Gezici and Şekeroğlu, 2019). The significant antileishmanial activity of *A. gracilis* ethyl acetate fraction indicates the presence of bioactive metabolites. Ferulic acid, a phenolic compound, was identified as the major active component. Its moderate inhibitory activity and strong docking interactions with GP63 suggest potential as a lead molecule.Compared with previous reports, phenolic acids such as caffeic and gallic acid have shown similar inhibition patterns against *Leishmania* species, suggesting a conserved mechanism of enzyme inhibition. The hydrogen bonding with catalytic residues (SER-234 and GLU-166) may disrupt the proteolytic activity of GP63, leading to parasite death.

Ferulic acid is widely reported to possess antimicrobial, antioxidant, and antiparasitic properties (Chaudhary et al., 2019; Ou et al., 2004; Zduńska et al., 2018). Its ability to interact with key amino acid residues of leishmanolysin may explain its biological effect. Previous studies have shown similar docking profiles of phenolic acids against parasitic enzymes, supporting its relevance as a natural inhibitor (Belkhelfa-Slimani and Djerdjouri, 2017; Carter et al., 2021; Garcia et al., 2019; Monzote et al., 2016) Natural compounds are particularly valuable in neglected disease drug discovery due to their structural diversity and reduced toxicity (Nobili et al., 2009) . Further preclinical and *in vivo* studies are required to confirm its therapeutic efficacy and optimize its pharmacological profile.

5. Conclusion

Ferulic acid isolated from *Asparagus gracilis* exhibited moderate antileishmanial activity against *L. tropica* and strong molecular docking affinity to GP63 protease. These findings support the potential of ferulic acid as a natural lead compound for developing novel antileishmanial agents.

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