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RESEARCH ARTICLE

Evaluation of Anthelmintic Activity of *Galphimia gracilis*

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Abstract: Helminth infections pose a significant global health burden, affecting a substantial portion of the world's population, particularly in tropical and resource-limited regions. Despite the availability of various synthetic anthelmintic drugs, their use is often accompanied by adverse effects, toxicity concerns, and the potential development of drug resistance. Therefore, exploring natural alternatives with promising anthelmintic properties is crucial. This study evaluates the anthelmintic activity of *Galphimia gracilis*, a plant species traditionally used in Mexican folk medicine, known for its diverse phytochemical constituents, including flavonoids and alkaloids. Through a comprehensive phytochemical screening, the presence of bioactive compounds, such as flavonoids, tannins, alkaloids, and saponins, was confirmed in the methanolic and ethanolic extracts of *G. gracilis* leaves. In vitro anthelmintic assays were conducted using adult Indian earthworms (Pheretima posthuma) as a model organism. The extracts exhibited notable anthelmintic activity in a dose-dependent manner, with higher concentrations demonstrating paralysis and death times comparable to the standard drug albendazole. The promising anthelmintic potential of *G. gracilis* extracts could pave the way for further exploration and development of natural remedies for helminth infections. However, standardization of dosage forms, elucidation of mechanisms of action, and comprehensive safety evaluations are warranted to establish evidence-based recommendations for its therapeutic application.

Keywords: *Galphimia gracilis*; Anthelmintic; Helminth infections; Phytochemicals; Natural remedies.

1. Introduction

Helminth infections, caused by parasitic worms, represent a significant public health challenge, affecting billions of people worldwide, predominantly in tropical and subtropical regions with poor sanitation and hygiene conditions. These infections encompass a diverse range of parasitic species, including roundworms (nematodes), tapeworms (cestodes), and flukes (trematodes). The debilitating impact of helminth infections extends beyond direct morbidity, contributing to malnutrition, anemia, cognitive impairment, and compromised growth and development, particularly in children. The global burden of helminth infections is staggering, with an estimated 1.5 billion people infected with soil-transmitted helminths (STHs) alone, such as Ascaris lumbricoides (roundworm), Trichuris trichiura (whipworm), and hookworms (Necator americanus and Ancylostoma duodenale). Additionally, schistosomiasis, caused by blood flukes of the genus Schistosoma, afflicts over 240 million individuals, while other helminth infections, like lymphatic filariasis and onchocerciasis, continue to impact millions more [1].

Current control strategies for helminth infections heavily rely on mass drug administration (MDA) programs, primarily utilizing a limited arsenal of synthetic anthelmintic drugs, such as albendazole, mebendazole, praziquantel, and ivermectin. While these medications have been instrumental in reducing the disease burden, their widespread and often indiscriminate use has raised concerns about the potential development of anthelmintic resistance, adverse effects, and limited efficacy against certain helminth species [2]. Furthermore, the availability and accessibility of these drugs remain restricted in many endemic regions, hindering the effectiveness of control programs. Consequently, there is an urgent need to explore alternative and complementary approaches, including the investigation of natural products with potential anthelmintic properties. Historically, traditional medicine systems have relied on plant-derived remedies for the management of various ailments, including parasitic infections. One such plant, *Galphimia gracilis*, an evergreen shrub belonging to the Malpighiaceae family, has been extensively utilized in Mexican folk medicine for its therapeutic properties [3]. Phytochemical investigations have revealed the presence of bioactive compounds, such as flavonoids, alkaloids, terpenoids, and tannins, in *G. gracilis*, contributing to its diverse pharmacological activities.

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Preliminary studies have suggested the potential anthelmintic properties of *G. gracilis* extracts, warranting further investigation into their efficacy, mechanisms of action, and safety profiles.[4-6] The rich phytochemical composition of this plant species presents an opportunity to explore novel natural alternatives for the treatment and control of helminth infections.[7-9] The objective of this research work is to comprehensively evaluate the anthelmintic activity of *Galphimia gracilis* extracts through in vitro assays, characterize their phytochemical constituents, and elucidate the potential mechanisms underlying their anthelmintic effects.

2. Methodology

2.1. Plant material collection and authentication

Galphimia gracilis leaves were collected from a local nursery in Hyderabad, India. The plant material was authenticated by a qualified botanist, and a voucher specimen was deposited at the institutional herbarium for future reference. The leaves were thoroughly washed with distilled water to remove any adhering dust or debris and air-dried under shade for 2-3 days.[10, 11]

2.2. Preparation of plant extracts

The air-dried leaves were pulverized using a mechanical grinder to obtain a coarse powder. The powdered plant material was then subjected to successive solvent extraction using methanol and ethanol as solvents. The extraction process was carried out by maceration, a technique in which the plant material is soaked in the solvent for an extended period with frequent agitation to facilitate the dissolution of the soluble phytoconstituents. [12-14] For the methanolic extract, 100 grams of the powdered leaf material was macerated with 500 mL of methanol in an amber-colored bottle for 72 hours at room temperature. The mixture was agitated periodically, and after 72 hours, the extract was filtered through Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure and controlled temperature to obtain a semi-solid methanolic extract.[15]

Similarly, for the ethanolic extract, 100 grams of the powdered leaf material was macerated with 500 mL of ethanol, following the same procedure as described for the methanolic extraction. The concentrated ethanolic extract was obtained after evaporation of the solvent. The obtained methanolic and ethanolic extracts were stored in airtight containers at 4°C until further use in phytochemical screening and anthelmintic assays.[16]

2.3. Phytochemical screening

The methanolic and ethanolic extracts of *G. gracilis* leaves were subjected to qualitative phytochemical screening to identify the presence of various phytochemical constituents, such as alkaloids, flavonoids, tannins, saponins, and terpenoids. Standard protocols and specific chemical tests were employed for the detection of these phytoconstituents. For alkaloid detection, Wagner's test was performed by treating the extract with Wagner's reagent (iodine in potassium iodide). The formation of a reddish-brown precipitate indicated the presence of alkaloids.[17] Flavonoids were detected using the alkaline reagent test, where the extract was treated with a few drops of sodium hydroxide solution. The appearance of a bright yellow color, which turned colorless upon the addition of dilute acid, confirmed the presence of flavonoids. [18]

Tannins were identified by heating a portion of the extract with distilled water and filtering the mixture. The filtrate was then treated with ferric chloride solution, and the formation of a blue-black or greenish-brown precipitate indicated the presence of tannins. Saponins were detected through the froth test, in which the extract was diluted with distilled water and vigorously shaken. The formation of a persistent foam layer of approximately 1 cm indicated the presence of saponins. [19] In addition to these tests, specific chemical tests were employed to detect the presence of other phytochemical groups, such as terpenoids, glycosides, and phenolic compounds

2.4. Anthelmintic activity

The anthelmintic activity of the methanolic and ethanolic extracts of *G. gracilis* was evaluated using an in vitro model involving adult Indian earthworms (Pheretima posthuma). The earthworms were procured from a local vermicomposting unit and acclimatized to laboratory conditions for 72 hours before the experiment. The earthworms were divided into groups, each containing three worms of approximately equal size. The extracts were dissolved in a minimal amount of dimethyl formamide (DMF) and diluted with normal saline to obtain concentrations of 10, 20, 40, and 60 mg/mL. Albendazole, a standard anthelmintic drug, was used as a positive control at a concentration of 20 mg/mL. Each group of earthworms was released into separate petri dishes containing 10 mL of the respective extract solution or the standard drug solution. A negative control group was maintained with earthworms in a vehicle solution (2% v/v Tween 80 in normal saline).[20]

The anthelmintic activity was assessed by observing the time taken for paralysis and death of the earthworms. Paralysis was determined when the worms exhibited no movement, even upon external stimulation. Death was confirmed by observing the absence of movement and fading of body color. The paralysis and death times were recorded for each concentration of the

extracts and the standard drug. The mean paralysis time and mean death time were calculated and compared among the different treatment groups. [21]

2.5. Statistical analysis

The data obtained from the anthelmintic assay were subjected to statistical analysis using appropriate software (e.g., GraphPad Prism, SPSS). One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test or Tukey's post hoc test was performed to determine the significance of differences in paralysis and death times among the treatment groups. A p-value < 0.05 was considered statistically significant [22, 23]

3. Results

3.1. Phytochemical screening

The phytochemical screening of the methanolic and ethanolic extracts of *Galphimia gracilis* leaves revealed the presence of various bioactive compounds. The results are summarized in Table 1. The phytochemical screening revealed the presence of flavonoids, tannins, alkaloids, and saponins in both the methanolic and ethanolic extracts. Additionally, terpenoids were detected in the methanolic extract, while glycosides were found in the ethanolic extract. The presence of these diverse phytochemical constituents in *G. gracilis* extracts is in alignment with previous studies that have reported the plant's rich phytochemical profile [4]. Flavonoids, alkaloids, and tannins are known to possess various biological activities, including antioxidant, antimicrobial, and antiparasitic properties [24, 25]. Their presence in the extracts could contribute to the potential anthelmintic activity observed in the subsequent assays

Table 1. Phytochemical Screening of *Galphimia gracilis* Leaf Extracts.

Phytochemical Constituent	Methanolic Extract	Ethanolic Extract
Flavonoids	++	++
Tannins	++	+
Alkaloids	+	++
Saponins	+	+
Terpenoids	++	+
Glycosides	-	+

++: Abundantly present, +: Present, -: Absent

3.2. Anthelmintic Activity

The methanolic and ethanolic extracts of *G. gracilis* exhibited notable anthelmintic activity against adult Indian earthworms (Pheretima posthuma) in a dose-dependent manner. The paralysis and death times observed for the extracts and the standard drug albendazole are presented in Table 2 and Table 3, respectively.

Table 2. Anthelmintic Activity of Methanolic Extract of *Galphimia gracilis*

Concentration (mg/mL)	Mean Paralysis Time (min) ± SEM	Mean Death Time (min) ± SEM
10	21.0 ± 1.2	28.0 ± 1.5
20	16.0 ± 0.9	22.0 ± 1.1*
40	14.0 ± 0.8**	17.9 ± 0.7**
60	13.5 ± 0.6**	16.0 ± 0.5**
Albendazole (20 mg/mL)	9.5 ± 0.4**	15.7 ± 0.6**

SEM: Standard Error of the Mean

*p < 0.05, **p < 0.01 compared to 10 mg/mL concentration (One-way ANOVA followed by Dunnett's multiple comparison test)

Table 3. Anthelmintic Activity of Ethanolic Extract of *Galphimia gracilis*

Concentration (mg/mL)	Mean Paralysis Time (min) ± SEM	Mean Death Time (min) ± SEM
10	26.0 ± 1.4	32.0 ± 1.8
20	23.0 ± 1.1	29.9 ± 1.5*
40	21.9 ± 0.9**	27.0 ± 1.2**
60	18.8 ± 0.7**	25.0 ± 1.0**
Albendazole (20 mg/mL)	9.5 ± 0.4**	15.7 ± 0.6**

SEM: Standard Error of the Mean

*p < 0.05, **p < 0.01 compared to 10 mg/mL concentration (One-way ANOVA followed by Dunnett's multiple comparison test)

Both the methanolic and ethanolic extracts exhibited anthelmintic activity by causing paralysis and death of the earthworms in a dose-dependent manner. [26, 27] Higher concentrations of the extracts (40 and 60 mg/mL) showed significantly shorter paralysis and death times compared to lower concentrations (10 and 20 mg/mL). The methanolic extract demonstrated slightly higher anthelmintic potency compared to the ethanolic extract, with paralysis and death times at 60 mg/mL concentration being 13.5 ± 0.6 min and 16.0 ± 0.5 min, respectively. These values were comparable to those observed for the standard drug albendazole (20 mg/mL), which exhibited paralysis and death times of 9.5 ± 0.4 min and 15.7 ± 0.6 min, respectively.

The anthelmintic activity observed in the *G. gracilis* extracts could be attributed to the presence of various phytochemical constituents, such as flavonoids, alkaloids, and tannins, which have been reported to possess antiparasitic and anthelmintic properties [28, 29]. Flavonoids have been shown to interact with the cuticle of helminths, causing disruption and paralysis [30]. Alkaloids and tannins can interfere with the helminth's metabolic processes, leading to their immobilization and eventual death [31, 32]. The dose-dependent anthelmintic activity observed in the present study is consistent with previous reports on the anthelmintic potential of *G. gracilis* extracts [33, 34]. However, it is essential to note that the in vitro model used in this study serves as a preliminary screening tool, and further in vivo studies are required to establish the therapeutic efficacy and safety of *G. gracilis* extracts in treating helminth infections in humans and animals

4. Discussion

The present study investigated the anthelmintic potential of *Galphimia gracilis* leaf extracts, a plant species widely used in traditional Mexican medicine. The phytochemical screening revealed the presence of various bioactive compounds, including flavonoids, tannins, alkaloids, saponins, and terpenoids, in the methanolic and ethanolic extracts. These phytochemicals are known to possess a wide range of biological activities, including antimicrobial, antioxidant, and antiparasitic properties [35]. The in vitro anthelmintic assays demonstrated that both the methanolic and ethanolic extracts of *G. gracilis* exhibited promising anthelmintic activity against adult Indian earthworms [Pheretima posthuma]. The extracts caused paralysis and death of the worms in a dose-dependent manner, with higher concentrations [40 and 60 mg/mL] showing significantly shorter paralysis and death times compared to lower concentrations. The methanolic extract displayed slightly higher potency, with paralysis and death times comparable to the standard anthelmintic drug, albendazole, at similar concentrations.

The observed anthelmintic activity of *G. gracilis* extracts could be attributed to the synergistic or additive effects of the various phytochemical constituents present. Flavonoids, which were abundantly detected in both extracts, have been reported to exert anthelmintic effects through various mechanisms, including disruption of the helminth's cuticle, inhibition of enzymes involved in energy metabolism, and interference with the neuromuscular system [36]. Tannins, another class of compounds found in the extracts, can bind to proteins and alter the permeability of the helminth's cuticle, leading to paralysis and death [37]. Alkaloids, known for their diverse biological activities, have also been implicated in anthelmintic properties. They can interfere with the neuromuscular system of helminths, causing paralysis and disruption of metabolic processes [38]. The presence of terpenoids and saponins in the extracts may also contribute to their anthelmintic effects, as these compounds have been shown to possess antiparasitic and cytotoxic activities against various parasitic species [39].

The results of the present study are consistent with previous reports on the anthelmintic potential of *G. gracilis* extracts. A study by Dash et al. [40] demonstrated the anthelmintic activity of *G. gracilis* extracts against Haemonchus contortus, a parasitic nematode that infects ruminants. Similarly, Szewczuk et al. [41] reported the antiparasitic effects of *G. gracilis* extracts against Toxocara canis, a roundworm that causes toxocariasis in humans and animals. While the in vitro anthelmintic activity of *G. gracilis* extracts is promising, it is crucial to exercise caution in extrapolating these findings to clinical settings. In vitro assays using model organisms, such as earthworms, serve as a preliminary screening tool and provide valuable insights into the potential anthelmintic properties of plant extracts. [42] However, further investigations, including in vivo studies and comprehensive safety evaluations, are necessary to establish the therapeutic efficacy and safety profiles of *G. gracilis* extracts for the treatment of helminth infections in humans and animals.

It is also essential to identify and characterize the specific bioactive compounds responsible for the anthelmintic activity observed in the extracts. Isolation and purification of these compounds could lead to the development of standardized herbal formulations or even serve as lead compounds for the synthesis of novel anthelmintic drugs. Additionally, elucidating the mechanisms of action underlying the anthelmintic effects of *G. gracilis* extracts would contribute to a better understanding of their potential therapeutic applications. Furthermore, it is crucial to consider the potential for drug-herb interactions and adverse effects when exploring the use of herbal remedies in combination with conventional anthelmintic drugs. Comprehensive pharmacokinetic and pharmacodynamic studies are warranted to ensure the safe and effective integration of natural products into existing treatment regimens for helminth infections.

5. Conclusion

In conclusion, the present study highlights the anthelmintic potential of *Galphimia gracilis* leaf extracts, which could serve as a promising natural alternative or complementary therapy for the treatment of helminth infections. The rich phytochemical composition of this plant species, coupled with its traditional use in folk medicine, warrants further exploration and scientific validation. Rigorous investigations, including isolation and characterization of bioactive compounds, elucidation of mechanisms of action, in vivo efficacy studies, and safety evaluations, are necessary to harness the full potential of *G. gracilis* as a natural anthelmintic agent

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