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Research Article

Antidiabetic Potential of *Loranthus longiflorus* Desr. from *Azadirachta indica* and *Albizzia lebbeck* Host Trees: A Cultural Parasitic Medicinal Plant from the Jammu Foothills of Azad Jammu and Kashmir, Pakistan

Muhammad Waqas Mazhar*[1], Muhammad Ishtiaq[2], Mehwish Maqbool[1], and Muhammad Zaheer Ahmad[3]

[1]Department of Botany, Mirpur University of Science and Technology, Mirpur 10250, Pakistan.
[2]Department of Botany, Climate Change Research Centre, Herbarium and Biodiversity Conservation, Azad Jammu and Kashmir University of Bhimber (AJKUoB), Bhimber-10040 (AJK), Pakistan.
[3]Department of Botany, The University of Lahore, Lahore, Pakistan.
*Correspondence: mwmazhar403@gmail.com

Abstract

This study explores the comparative antidiabetic potential of ***Loranthus longiflorus*** from two different hosts, ***Azadirachta indica*** and ***Albizzia lebbeck***, in an alloxan-induced diabetic rabbit model. Before assessing its antidiabetic efficacy, the plant's ethnobotanical uses and qualitative phytochemical profile were investigated, revealing alkaloids, saponins, and flavonoids as major secondary metabolites, which were comparatively higher in ***L. longiflorus*** taken from ***A. lebbeck*** than ***A. indica***. Methanolic leaf and bark extracts were administered orally to diabetic rabbits for 30 days, with blood glucose levels, body weights, and serum insulin profiles evaluated across four groups: normal control (NCG), diabetic control (DCG), treatment control (TCG) with glibenclamide (0.2 mg/kg), and trial group (TG). A 1000 mg dose of ***L. longiflorus*** leaf extract from ***A. lebbeck*** showed the best antidiabetic activity, reducing body weight and blood glucose levels by 14.74% and 76.43% on day 30, compared to 11.06% and 64.20% reductions with the same dose from ***A. indica***. Additionally, the 1000 mg dose from both hosts significantly elevated serum insulin profiles ($p < 0.001$). The findings suggest that ***Loranthus longiflorus***, particularly when derived from ***Albizzia lebbeck***, holds significant antidiabetic potential, likely due to its rich phytochemical composition. These results underscore the importance of host-tree influence on the therapeutic efficacy of parasitic plants.

Keywords: Diabetes mellitus, Traditional ethnomedicines, *Loranthus longiflorus*, methanolic extract, Hyperglycaemia, Serum insulin

1. Introduction

Diabetes is a chronic metabolic disorder characterized by elevated blood glucose levels, either due to insufficient insulin production by the pancreas or the body's inability to use insulin effectively. Unmanaged diabetes can lead to serious complications, including cardiac disease, kidney failure, nerve damage, and vision loss. Effective management of diabetes typically involves lifestyle modifications, medications, and in some cases, insulin therapy, although these approaches often come with challenges and side effects (Mushtaq et al. 2024).

Various treatments and remedial protocols for diabetes are available globally. These measures include insulin injections, oral glucophage medications, stem cell therapy, and islet transplantation (Kundo et al. 2021). However, transplantation carries a significant risk of immune rejection of foreign tissues by the patient's body. Moreover, transplantation is highly expensive, and its acces-

sibility in developing and underdeveloped countries remains uncertain (Liu et al. 2023). Regularly using insulin injections requires strict adherence to schedules, which can be burdensome. Additionally, many individuals who rely on insulin injections experience psychological distress and anxiety. Oral glucose-lowering medications, while convenient, often cause adverse side effects. Prolonged use of these drugs can lead to chronic stomach and kidney infections (Tran, Pham, and Le 2020).

Due to these challenges, many diabetes patients are hesitant to rely on conventional treatments and are increasingly turning to herbal medicines and traditional medicinal plants. This growing preference stems from the reduced side effects associated with herbal remedies and the increasing recognition of their anti-hyperglycemic properties (Rahman et al. 2022). Advances in phytochemical profiling and purification technologies have further amplified the popularity of "green medicines" (GMs). Currently, phytochemicals are the basis for approximately 50–60% of medications used globally for treating and managing various diseases. Cultural medicinal plants are particularly advantageous, being cost-effective, widely available to Indigenous populations, and typically associated with minimal or no side effects (Rahman et al. 2022, Lalitha et al. 2020).

This study aimed to investigate the potential of herbal medicines for mitigating diabetes mellitus, focusing on *Loranthus longiflorus*, a phytochemically rich parasitic plant. *Loranthus longiflorus* Desr., belonging to the Loranthaceae family (commonly known as the mistletoe family), is recognized worldwide for its parasitic mode of nutrition (Rahman et al. 2022, Lalitha et al. 2020). This plant is cosmopolitan in its distribution and parasitizes a wide range of hosts, including *Albizzia lebbeck* (Fabaceae), *Azadirachta indica* (Meliaceae), and other trees. It forms a primary haustoria structure that penetrates the cortical and epicortical regions of the host plants to extract nutrients.

In Azad Jammu and Kashmir (AJK), the plant is culturally known as "*parakh*" and is commonly found on trees growing in the foothills of Jammu (Khanum et al. 2024). Traditional knowledge suggests that the phytochemical profile of a parasitic plant reflects that of its host (Piwowarczyk et al. 2020). Among local communities in AJK and the Jammu foothills, extracts of *L. longiflorus* derived from *A. lebbeck* are considered particularly effective, with people frequently using these extracts over those derived from other hosts (Khanum et al. 2024).

With this background, our study was designed to evaluate the antidiabetic potential of *L. longiflorus* obtained from two prominent host plants in the study area, *A. lebbeck* and *A. indica.* Our hypothesis posited that methanolic extracts of *L. longiflorus* from *A. lebbeck* would demonstrate greater antidiabetic efficacy compared to extracts from *A. indica.*

The primary objective of this research was to investigate the antidiabetic properties of methanolic extracts of *L. longiflorus*, specifically comparing the effects of extracts obtained from *A. lebbeck* and *A. indica.* Additionally, the study aimed to explore the plant's potential as a novel GM for diabetes management. By addressing these objectives, we seek to provide valuable insights into the medicinal properties of *L. longiflorus*, emphasizing the influence of different host plants on its phytochemical composition and therapeutic efficacy.

2. Materials and Methods

2.1. Collection of Plant Materials and Ethical Permissions

Loranthus longiflorus plants were collected from the tree species *Azadirachta indica* and *Albizzia lebbeck* from the Jammu foothills (Mughal et al. 2017). The sample collection was completed in March 2023. Gloves and sterilized tools were used to handle the plant material to prevent contamination. Dirt and debris were removed gently by shaking and brushing. The collected plant parts were air-dried in a well-ventilated, shaded area to preserve

bioactive compounds, avoiding direct sunlight. The drying process was monitored regularly to ensure uniformity and prevent any pathogenic contamination. Once dried to a crisp consistency, the plant material was stored in airtight, labelled containers in a cool, dark, and dry location. The plant samples were identified by taxonomist Dr. Muhammad Ishtiaq, Professor at the Department of Botany, AJK, and were cross-checked from the Flora of Pakistan. The samples were dried and mounted on herbarium sheets deposited in the herbarium of the Department of Botany Mirpur University of Science and Technology (MUST), AJK under voucher number MUH-889 (Khanum et al. 2024).

The research project received official permission and approval from the Departmental Ethics Committee (DEC) through an official correspondence (Reference Number: 292/DEC/BOT/2023; Date: 16/02/2023), which was endorsed by the university department head. Adhering to ethical standards and regulatory protocols, the experimental protocol for our field research, encompassing plant collection and animal trials, underwent proper scrutiny and was duly sanctioned by the DEC. The pertinent researchers, students, and field workers meticulously adhered to the guidelines provided by forest officers. Every participant was furnished with informed consent, and their rights, privacy, and confidentiality were rigorously safeguarded throughout the entire study. Moreover, all research activities were executed in strict accordance with applicable national laws and international standards that oversee plant research. To ensure complete compliance with legal and ethical frameworks, the requisite permits or approvals were secured from relevant authorities. Our experimental research on animals and field studies on plants were conducted meticulously, adhering to the guidelines and legislation stipulated by institutional, national, and international regulatory bodies. This approach guaranteed the ethical and legal integrity of the entire research process (Khanum et al. 2024).

2.2. Ethnomedicinal Studies

In the present study, the experimental trials were conducted employing the EPA model, encompassing ethnobotanical survey (E), phytochemical profiling (P), and activity assessment (A). For ethnomedicinal studies of the plant being used as a traditional GM, interviews were conducted based on open-ended and close-ended questionnaires following protocols as described (Amjad et al. 2016). The opinions of Indigenous people were recorded by random and planned field visits across rural areas of Jammu Foothills, AJK. Local translator services were hired for assistance in the interviews. The ethnomedicinal data about the plant were gathered from 100 informants, comprising 60 males and 40 females. The people were asked about the use of the vernacular name for *Loranthus longiflorus*, its host species, the medicinal use of the plant from a particular host, and its use as medicine. Furthermore, the use of the plant in traditional recipes and the occurrence of the plant across various places were studied. The results were organized and authenticated using the informant consensus factor (ICF) and fidelity level (FL) approach (Ishtiaq et al. 2012, Ajaib et al. 2022). FL is an index used that describes unanimous witnesses of informants on the 'utilization of a specific plant for specific use or purpose'. The FL is an indicator of the popularity of specific plants as herbal drugs that are prevalently used to treat specific diseases or for any other purpose in the research area. The FL was calculated using equations extracted from previous work as described (Ishtiaq et al. 2012, Ajaib et al. 2022).

$$FL = Np/N \times 100$$

where FL is represented in %; Np is the number of respondents who claim "specific use of a particular plant" for cure of a particular ailment or use for another purpose"; and N describes the "total number of respondents" who use the plant for curing a particular disease or for any other use in the study area.

Table 1: Traditional Use of *L. longiflorus* as a green medicine from the Jammu foothills of AJK, Pakistan

S. N	Disease cured	Part used	Recipe
1.	Diabetes	Leaf	Leaf-crushed powder is taken with milk twice a day.
2.	Menstrual disorders	Leaf	The leaf of the plant is crushed and the extract mixed
3.	Pulmonary tuberculosis and asthma	Leaf	The extract is used with honey for one week.
4.	Cancer, tumors	Leaves	Crude extract.
5.	Aphrodisiac	Leaf and Bark	Extract or juice of the plant, combined with a little honey and other medicinal herbal extracts, acts as an aphrodisiac.
6.	Renal and vesical calculi	Leaf and flower	Crude extract of leaf and flower is effect tonic when taken with honey.

The informant consensus factor **(**ICF) index was calculated by the method as described by early researchers (Ishtiaq et al. 2012, Ajaib et al. 2022).

$$ICF = n_{ur} - n_t / n_{ur} - 1$$

where n_{ur} represents the "number of uses of a medicinal plant" for each category and it is the "total number of plant species used" to treat the particular group of infirmity or ailments.

2.3. Phytochemical Profiling

Fifty grams of powdered leaves, flowers, and bark were each mixed with 250 mL of methanol in separate containers to prepare a methanolic extract. The mixture was left at room temperature for seven days with intermittent shaking to facilitate the extraction of bioactive compounds. Afterward, the extract was filtered through Whatman No. 1 filter paper, and the filtrate was collected for further analysis. For alkaloids, the Mayer and Wagner tests were conducted (Harborne 1998). Flavonoids were assessed through the Ferric Chloride and Alkaline Reagent tests (Evans 2002). Protein content was determined using the Millon and Biuret tests, indicating differences among plant parts and host species (Plummer 1978). Saponins were analyzed with the Foam and Bromine Water tests, revealing notable variations (Obadoni and Ochuko 2002). Tannins were assessed using the Ferric Chloride Test (Evans 2002), while carbohydrates were evaluated through the Barfoed and Benedict tests, each showing distinct patterns (Harborne 1998). Triterpenoids were examined via the Salkowaski Test (Sofowora 1996), and cardiac glycosides were investigated using the Bromine Water Test (Evans 2009). These comprehensive tests provide a nuanced understanding of the phytochemical profiles of *L. longiflorus*, elucidating potential variations attributable to both host species and plant parts (Ishtiaq et al. 2012, Ajaib et al. 2022).

2.4. Antidiabetic Activity Assessment

The antidiabetic potential of *L. longiflorus* from two host tree species was evaluated by performing experimental trials on male rabbits (*Oryctolagus cuniculus* L.). Test animals were brought to the experimental area one week before the start of the experimental trials. The weights of the rabbits were measured. The rabbits weighed between 1.5 and 2 kg. Test animals were provided with green vegetables, grains, and grass as feed. Approval from the Departmental Ethics Committee, as guided by the University Ethical Committee for Animal Care, was obtained following national guidelines for animal care.

For the preparation of diabetic model animals, test animals were fasted for 6 hours before the induction of diabetes to ensure consistent baseline glucose levels. Before administering Alloxan, the fasting blood glucose levels of the animals were measured and recorded to enable comparison with post-induction glucose levels. The marginal

Table 2: Informant Consensus Factor (ICF) values for the *L. longiflorus* from the Jammu foothills of Azad Jammu and Kashmir, Pakistan.

S. No	*Disease category*	*Species(nt)*	*All Species (%)*	*Use Citation (nur)*	*Use Citation (%)*	*ICF=nur-nt/nur-1*
1	Diabetes	3	60	15	75	13.8
2	Wound healing	4	80	13	65	11.8
3	Respiratory disease	2	40	12	60	10.6
4	Menstrual disorders	4	80	11	55	9.6
5	Renal and Vesical calculi	1	20	3	15	1.7
6	Ulcers	2	40	7	35	5.7
7	Cancer, tumours	2	40	7	35	5.7

Table 3. Fidelity Levels of *Loranthus longiflorus* used to cure diabetes mellitus from the Jammu foothills, AJK.

Plant	*Host*	*Np*	*N*	*F.L%=NP/N×100*
Loranthus longiflorus	*Albizia lebbeck*	71	100	71
	Azadirachta indica	52	94	55.35
	Acacia modesta	41	76	53.94

ear vein of the test animals was then prepared by applying xylene to dilate the vein and facilitate the injection. Lignocaine was applied to the injection site to reduce the pain sensation. Alloxan monohydrate was dissolved in saline solution at a dose of 90 mg/kg, which was found to be effective for inducing diabetes in the test animals. The freshly prepared Alloxan solution was primed and immediately injected into the marginal ear vein. Following the injection, the blood glucose levels of the animals were monitored, and experimentation proceeded once their glucose levels were confirmed to have reached 300 mg/dL, following the protocols described by Mushtaq et al. (Mushtaq et al. 2016).

Four major test groups were designed and named the normal control group (NCG), disease control group (DCG), treatment control group (TCG), and trial group (TG).

- Group-I: The NCG group included three rabbits with normal/control sugar levels and without any treatment; these were considered nondiabetic.
- Group-II: DCG refers to a group in which diabetes is induced by alloxan but not treated by any other agent.
- Group III: TCG, in which diabetes was induced by an alloxan dose and treated with the commercially available medicine glibenclamide at a ratio of 0.2 mg/kg body weight.
- Group IV: Eight sub-groups of TG (T1 to T8) were subjected to different experimental trials, depending on the dose, host species, and plant part variation. T1 involved *A. indica* as the host species, with leaf material used for methanolic extracts, administered orally at a dose of 500 mg/kg body weight. T2 also utilized *A. indica* as the host species but with leaf material used for methanolic extracts at a higher dose of 1000 mg/kg body weight, administered orally. T3 and T4 both involved *A. indica*, with bark material used for methanolic extracts. T3 received an oral dose of 500 mg/kg body weight, while T4 received a higher dose of 1000 mg/kg body weight. Similarly, T5 to T8 involved *A. lebbeck* as the host species. In T5 and T6, leaf material was used for methanolic extracts, with doses of 500 mg/kg and 1000 mg/kg body weight, respectively, administered orally. T7 and T8 used bark material, with oral doses of 500 mg/kg and 1000 mg/kg body weight, respectively.

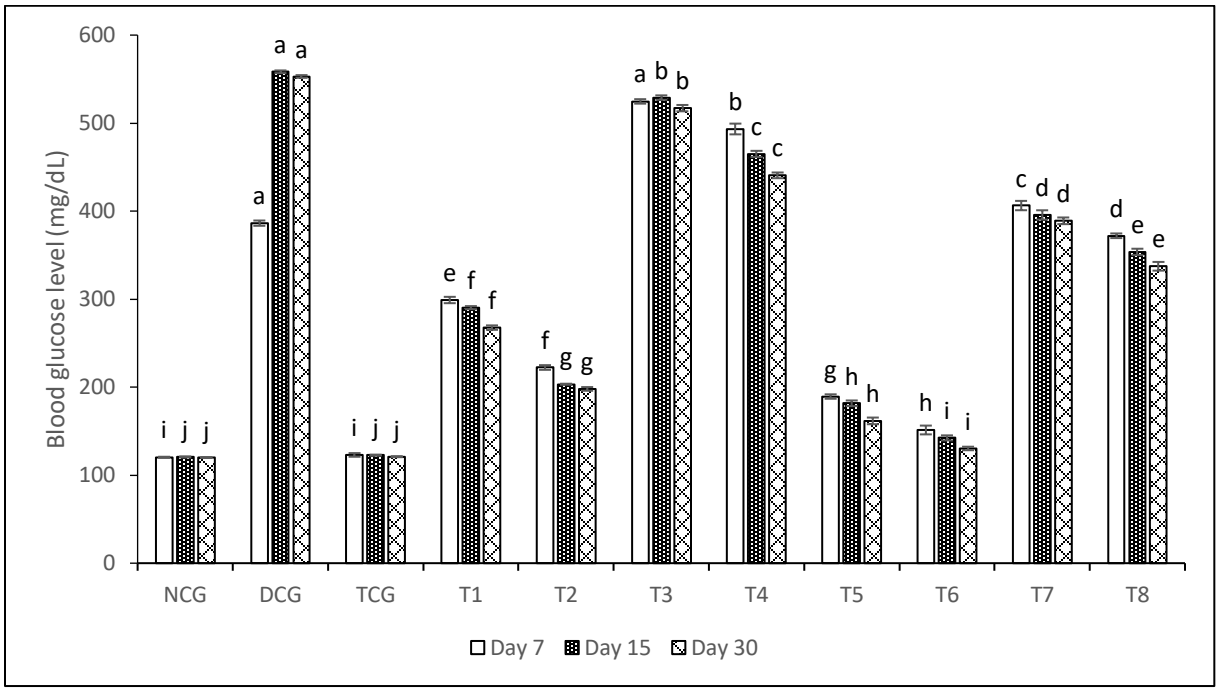

Figure 1. Bar charts depicting the mean data recorded on days 7, 15, and 30 during the experimental trial. The data is segregated using the LSD test (5%) and showcases the impact on blood glucose levels in alloxan-treated diabetic rabbit models. The treatment involves the use of bark and leaf extracts of *L. longiflorus* sourced from various host plants.

2.5. Diagnostic Test and Statistical Analysis

The blood glucose levels were recorded using a glucometer (ACCU-CHEK-active model glucometer; Roche Co.), and the glucose levels were measured on days 7, 15, and 30 of the experimental trial following the protocol adopted. Blood samples for measuring glucose levels were taken from the ears of the test animals, as alloxan was injected into the ears of the test animals. Serum insulin concentrations were measured by taking a blood sample at 3 cc. from the thigh veins of the test animals on the 15th and 30th days of the experimental trials. The sample was centrifuged at 1000 rpm as described by Mushtaq et al. (Mushtaq et al. 2016). The body weights of the test animals were monitored on days 7, 15, and 30 of the experimental trial using digital weighing machines as described (Khushk et al. 2010).

The collected data were organized into a matrix form and recorded on an Excel sheet, followed by analysis. A two-way analysis of variance (ANOVA) was conducted using Co-STAT (ver. 6.3, Cohort Software, Berkley, CA) at a significance level of 0.05. To differentiate means, the least significant difference (LSD 5%) was employed (Mazhar et al. 2023, Mazhar et al. 2024).

3. Results

The data obtained through the interview process revealed the potential use of *L. longiflorus* as an herbal medicine for managing several disorders. Traditionally, the plant has been used for managing diabetes, healing wounds, menstrual disorders, and treating respiratory problems such as asthma and pulmonary tuberculosis (Table 1). Furthermore, local hakims have utilized this woody hemi-parasitic shrub for treating vesical and renal calculi, as an aphrodisiac material, and as a cure for stomach ulcers and certain tumours.

The data presented in Table 2 indicate the results of the ICF from the people of the study area regarding the role of the plant in managing diabetes mellitus and other diseases. The highest value of 13.8 was obtained for the traditional use of the plant for

Table 4. Phytochemical profile of L. longiflorus from two hosts Azadirachta indica and A. lebbeck

S #	Phytochemicals	Tests Performed	Host *A. indica*			Host *A. lebbeck*		
			Leaf	Bark	Flower	Leaf	Bark	Flower
1.	Alkaloids	Mayer Test	+++	+	+	+++	+	+
		Wagner Test	++	+	+	++	+	+
2.	Flavonoids	Ferric Chloride Test	+	++	+	++++	++	+
		Alkaline Reagent test	++	++	+	+	++	+
3.	Proteins	Millon Test	+	++	+	+++	+	+
		Biuret Test	+	++	+	-	++	+
4.	Saponins	Foam test	+++	++	+	++++	++	+
5.	Tannins	Ferric chloride Test	+	++	+	++	+++	+
6.	Carbohydrates	Barfoed Test	+++	++	+	++	++	+
		Benedict Test	+	+	+	+	++	+
7.	Triterpenoids	Salkowaski Test	+++	+	+	+	++	+
8.	Cardiac glycoside	Bromine water Test	+++	++	+	+++	+++	+

(-): not detectable, (+): low quantity, (++): moderate quantity, (+++): high quantity, and (++++): highest quantity.

managing diabetes mellitus. Since the plant belongs to a wide range of hosts, FL values for the traditional use of plants as herbal remedies have been presented in Table 3. Based on the FL values, a phytochemical profile of the plant was constructed by taking samples from two main host species, *A. indica* and *A. lebbeck.*

Table 4 presents a detailed analysis of the phytochemical composition of *L. longiflorus* derived from two distinct host plants, *A. indica* and *A. lebbeck*, with a focus on various plant parts such as leaves, bark, and flowers. It is evident from the data that superior phytochemicals have been yielded through leaf tissues. Alkaloids, flavonoids, proteins, saponins, tannins, carbohydrates, triterpenoids, and cardiac glycosides were the key phytochemicals investigated, and specific tests were conducted to assess their presence. *L. longiflorus* from *A. indica* exhibited varying degrees of these phytochemicals across different plant parts, with the highest concentrations often observed in the leaves. On the other hand, *L. longiflorus* from *A. lebbeck* displayed a distinct pattern of phytochemical distribution, emphasizing the influence of the host species. The table shows that there is no variation in the alkaloid contents between the samples taken from two hosts of *L. longiflorus.* Flavonoid content is more pronounced in the leaves of *A. lebbeck*, where it reaches the highest levels. The leaves and bark of *A. indica* show moderate concentrations, while its flowers show a relatively lower concentration. In contrast, *A. lebbeck* shows high flavonoid content in the leaves, with moderate amounts in the bark and flowers. From both hosts methanolic extracts of the plant had moderate to high protein levels in their leaves and bark. Saponins are abundant in the leaves of both hosts, with a sample from *A. lebbeck* showing the highest levels. The plant extracts from both hosts had moderate tannin levels in their leaves, but *A. lebbeck* had higher levels in the bark. However, the tannins and triterpenoids contents of the sample from the hots *A. indica* were higher compared to *A. lebbeck.*

The current study suggested that leaf extract from *L. longiflorus* at a concentration of 1000 mg from the host *A. lebbeck* (T6) causes a maximum decrease in blood sugar levels in the experimental animals. A total of 500 mg of the bark extract from the host *A. indica* (T3) was the least effective (Figure 1). The leaf extracts (T1, T2, T5, and T6) better ameliorated hyper-glucose levels in rabbits than did the bark extracts from either host (T3, T4, T7, and T8). Similarly, the antidiabetic ability of *L. longiflorus* taken from the host *A. lebbeck* was better than that of the other host plant *A. indica.* By comparing the results of the treatment group 6

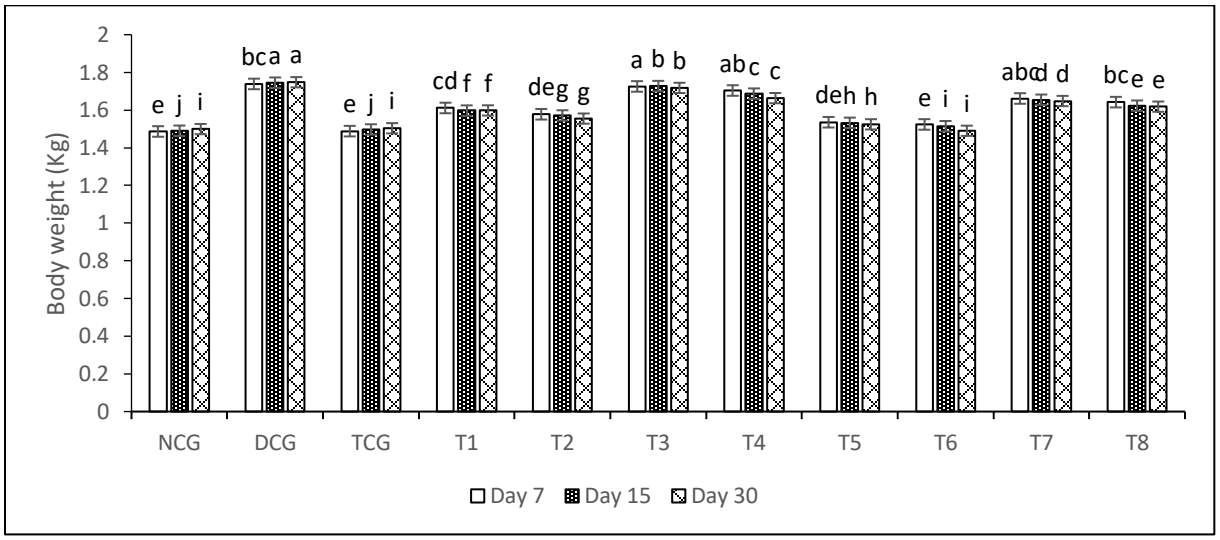

Figure 2. Bar charts depicting the mean data recorded on days 7, 15, and 30 during the experimental trial. The data is segregated using the LSD test (5%) and showcases the impact on body weights of alloxan-treated diabetic rabbit models. The treatment involves the use of bark and leaf extracts of *L. longiflorus* sourced from various host plants.

with those of the test animals treated with allopathic medicine (TCG), one can infer that the leaf extract of *L. longiflorus* at a concentration of 1000 mg produced results similar to those of the allopathic treatment group (Figure 1). Furthermore, it is clear from the data of the experimental trial that an increased duration of treatment leads to better results. These results were further strengthened through the time-by-treatment interaction results of the study as presented in table 5.

The percentage reduction in body weight (BW) and blood glucose levels compared to the DCG highlights the efficacy of methanolic extracts of *Loranthus longiflorus* derived from two host species, *Albizzia lebbeck* and *Azadirachta indica*. For BW, the DCG group had an average weight of 1.748 g. Treatment T1 (*A. indica*, 500 mg/kg) reduced body weight to 1.598 g, representing an 8.56% reduction compared to DCG. In comparison, T5 (*A. lebbeck*, 500 mg/kg) reduced body weight to 1.524 g, achieving a greater reduction of 12.79%. At the higher dose, T2 (*A. indica*, 1000 mg/kg) reduced body weight to 1.555 g, corresponding to an 11.02% reduction, while T6 (***A. lebbeck***, 1000 mg/kg) reduced body weight to 1.490 g, resulting in a 14.71% reduction.

The data presented in Figure 2 and Table 5 reflect the results of comparative studies of the weights of experimental animals treated with various medicaments of bark and leaf extracts obtained from *L. longiflorus*. A maximum decrease in body weights of rabbits was observed with treatment of 1000 mg from leaf extract of *L. longiflorus* belonging to host *A. lebbeck.* A minimum reduction in body weight was observed with 500 mg of bark extract from the host *A. indica*. Overall, the methanolic extracts obtained from *A. lebbeck* better reduced the body weights of the experimental animals. The data presented in Figure 2 suggest that leaf extract of *L. longiflorus* not only decreases elevated glucose levels but also helps to reduce body weight. For blood glucose levels, the DCG group had an average glucose level of 553 mg/dL. Treatment T1 (*A. indica*, 500 mg/kg) lowered glucose levels to 267.67 mg/dL, a 51.57% reduction compared to DCG. Meanwhile, T5 (*A. lebbeck*, 500 mg/kg) reduced glucose levels to 161.67 mg/dL, corresponding to a 70.76% reduction. At the higher dose, T2 (*A. indica*, 1000 mg/kg) decreased

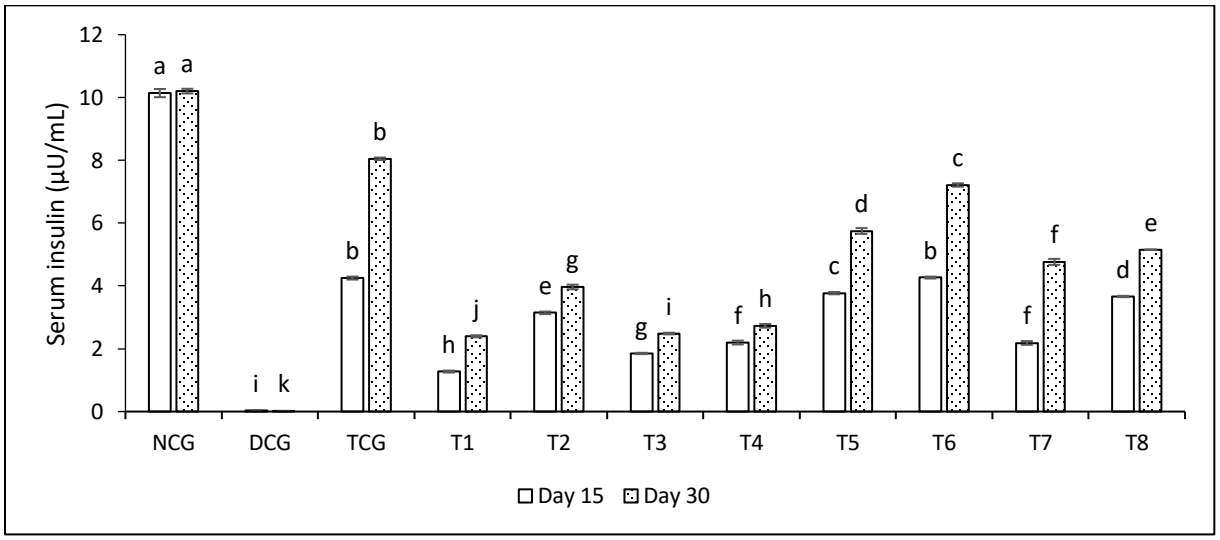

Figure 3. Bar charts depicting the mean data recorded on days 15, and 30 during the experimental trial. The data is segregated using the LSD test (5%) and showcases the impact on serum insulin levels of alloxan-treated diabetic rabbit models. The treatment involves the use of bark and leaf extracts of *L. longiflorus* sourced from various host plants.

glucose levels to 198 mg/dL, a reduction of 64.18%. In contrast, T6 (*A. lebbeck*, 1000 mg/kg) reduced glucose levels to 130.33 mg/dL, achieving a remarkable 76.44% reduction. The comparison of T1 and T5 at 500 mg/kg revealed that *A. lebbeck* (T5) had superior efficacy in reducing both body weight and blood glucose levels. Similarly, at the higher dose of 1000 mg/kg, T6 (*A. lebbeck*) outperformed T2 (*A. indica*) in both parameters. These results also show a dose-dependent response for both treatments, with the 1000 mg/kg dose producing greater reductions than the 500 mg/kg dose.

The methanolic extract from the leaf of *L. longiflorus* from the host *A. lebbeck* at a concentration of 1000 mg proved as the best treatment for boosting serum insulin concentrations (Figure 3). The bark extract treatment dose of 500 mg of *L. longiflorus* from the host *A. indica* had the least significant effect on increasing the serum insulin concentration. The comparison of serum insulin levels between treatments highlights the differences in efficacy of *L. longiflorus* extracts from *A. lebbeck*, and *A. indica* at different doses over time. On day 15, T5, which involved *A. lebbeck* leaf extract at 500 mg/kg, showed higher serum insulin levels (3.77) compared to T1, where *A. indica* leaf extract at the same dose resulted in a lower value of 1.27.

This indicates that T5 was more effective than T1 in improving serum insulin at this time point. At a higher dose of 1000 mg/kg, Treatment 6 (T6), which used *A. lebbeck* leaf extract, demonstrated a serum insulin level of 4.27 on day 15, surpassing Treatment 2 (T2), where *A. indica* leaf extract at the same dose resulted in a lower value of 3.14. Thus, T6 showed greater efficacy compared to T2 in enhancing serum insulin levels.

On **day 30**, a similar trend was observed. Treatment 5 (T5) recorded a serum insulin level of 5.75, which was notably higher than Treatment 1 (T1) at 2.40. This further confirms the superior efficacy of *A. lebbeck* leaf extract over *A. indica* leaf extract at the 500 mg/kg dose. Likewise, Treatment 6 (T6) achieved a serum insulin level of 7.21, outperforming Treatment 2 (T2), where the level was 3.97 at the same dose of 1000 mg/kg.

Overall, the results indicate that *L. longiflorus* extracts from *A. lebbeck* host plants exhibited better efficacy in improving serum insulin levels com

Table 5. Mean Square and p Values for time (days) by treatment interactions and individual factors via Two-Way ANOVA.

Variation Source	Degrees of freedom	Blood glucose levels
Treatment (Factor 1)	10	220961.77 *** (0.000)
Time (Factor 2)	2	1107.090 *** (0.000)
Interaction of treatments by time	20	3362.076 ns (0.184)
Error	66	2503.12
Variation Source	**Degrees of freedom**	**Body weights**
Treatment (Factor 1)	10	0.0759 *** (0.000)
Time (Factor 2)	2	0.0013 ***(0.000)
Interaction of treatments by time	20	0.000279 ***(0.000)
Error	66	0.000042
Variation Source	**Degrees of freedom**	**Serum insulin levels**
Treatment (Factor 1)	10	44.0280 *** (0.000)
Time (Factor 2)	1	35.280 ***(0.000)
Interaction of treatments by time	10	2.2860 ***(0.000)
Error	44	0.0027

***=significant at 0.001 levels; ns: non-significant

pared to those derived from *A. indica*. Furthermore, the higher dose (1000 mg/kg) consistently outperformed the lower dose (500 mg/kg), highlighting a dose-dependent effect on insulin improvement.

Table 5 presents the results of a two-way ANOVA conducted to analyze the effects of two factors, treatment (different doses of *L. longiflorus* from two hosts, including controls) and time (days 7, 15, and 30 for blood glucose and body weight; days 15 and 30 for serum insulin levels), as well as their interaction, on blood glucose levels, body weight, and serum insulin levels. For blood glucose levels, both treatment and time were found to have highly significant effects ($p < 0.001$). This indicates that the various doses of *L. longiflorus* significantly reduced blood glucose levels and that glucose levels also changed significantly throughout the study. However, the interaction between treatment and time was not significant ($p = 0.184$), suggesting that the treatment effects on blood glucose were consistent across all time points. The lack of interaction implies that the treatments exerted their hypoglycaemic effects independently of the day of measurement, making them reliably effective in lowering glucose levels over time.

For body weight, treatment, time, and their interaction all had highly significant effects ($p < 0.001$). This demonstrates that the treatments influenced body weight, and that weight changes occurred over time. Furthermore, the significant interaction effect indicates that the impact of treatments on body weight varied depending on the time point. This could reflect a dynamic physiological response to the treatments, where initial effects may differ from those observed at later stages of the trial. The time-dependent response emphasizes the importance of monitoring body weight throughout the treatment duration to fully understand its effects.

For serum insulin levels, all factors, including treatment, time, and their interaction, were highly significant (**$p < 0.001$**). The treatments significantly influenced insulin levels, demonstrating their potential to modulate this critical metabolic parameter. The significant time effect indicates that insulin levels naturally fluctuated between days 15 and 30, while the significant interaction suggests that

the treatments' effects on insulin were time-dependent. This could reflect a progressive improvement in insulin secretion or sensitivity, particularly in response to specific doses of ***L. longiflorus***. The interaction highlights the need for a nuanced approach to interpreting treatment outcomes at different stages of the trial.

4. Discussion

Loranthus longiflorus is a hemi-parasitic plant that attaches to various host species, including *A. lebbeck* and *A. indica*. Before investigating its potential antidiabetic activity, it was important to evaluate the ethnomedicinal significance of *L. longiflorus* in the study area. Furthermore, the phytochemical composition of *L. longiflorus* was assessed to identify the bioactive compounds that might contribute to its medicinal effects. This study focused on investigating the antidiabetic potential of *L. longiflorus*, with particular attention to the influence of its host species, *A. lebbeck* and *A. indica*. Different parts of the host plants—leaves and bark—were used to prepare the methanolic extracts, as these are commonly known to contain a range of bioactive compounds with potential antidiabetic effects (Ishtiaq et al. 2024, Sardar et al. 2023, Mushtaq et al. 2023). The interview data reveals insights into the potential therapeutic applications of *L. longiflorus*, showcasing its historical significance in traditional medicine (Table 1). The plant's traditional use in managing diabetes implies a role in regulating blood sugar levels, suggesting potential anti-diabetic properties (Khanum et al. 2024). Additionally, its application in wound healing suggests possible antimicrobial or tissue-regenerative capabilities, aligning with the plant's medicinal versatility (Lalitha et al. 2020). Moreover, the historical use of *L. longiflorus* for treating menstrual disorders and respiratory problems such as asthma and pulmonary tuberculosis implies a broader impact on reproductive and respiratory health. These findings not only emphasize the traditional knowledge surrounding the plant but also point toward its potential to address a range of health concerns (Khanum et al. 2024). These metrics provide insights into the prevalence and citation frequency of plant species across different disease categories, and the ICF values indicate the level of consensus among informants regarding the usage of these plants for specific health conditions (Tables 2 and 3). It is possibly because of the unique properties of *L. longiflorus*, such as its use as a cooling agent, diuretic remedy, and aphrodisiac. These findings are in good agreement with the previous works reported on *L. longiflorus* (Gouthaman et al. 2022, Lalitha et al. 2020).

Higher phytochemical yields were obtained through leaf extractions than from bark or flowers (Table 4). Leaves typically exhibit superior capabilities in yielding higher concentrations of phytochemicals, primarily attributed to their essential role in photosynthesis, the process through which sunlight is converted into chemical energy. The abundance of chloroplasts in leaf cells facilitates the efficient synthesis of various secondary metabolites. Given their metabolic activity, leaves function as central hubs for both the production and storage of phytochemicals (Maxiselly et al. 2022), encompassing alkaloids and flavonoids, which play integral roles in the plant's defense mechanisms against herbivores and pathogens. An examination of the phytochemical composition of *L. longiflorus* from different hosts suggested that extracts from *A. lebbeck* had higher contents compared to those from *A. indica*. These results are according to our hypothesis and are supported by FL analysis. This variation in the phytochemical profile of *L. longiflorus* may arise from differences in its host plants (Ajithkumar, Thomas, and Mathew 2021). *L. longiflorus* possesses specialized structures known as haustoria, allowing direct access to the vascular system of host plants. This direct connection likely facilitates the absorption of a diverse array of nutrients, including phytochemicals, from the host, potentially resulting in elevated concentrations of these compounds within the parasitic plant. Parasitic

plants often exhibit selectivity in absorbing specific compounds from the host, influenced by their nutritional and defense requirements. This selectivity can lead to the accumulation of particular phytochemicals that contribute to the parasitic plant's growth, development, and defense mechanisms. Some parasitic plants release chemical signals or exudates that can influence the metabolic processes of the host plant. It is plausible that the distinctive phytochemical profile of *L. longiflorus* is linked to variations in its host plants. Similar work has been reported on *Viscum album* (Santalaceae), another mistletoe (Majeed et al. 2021). The presence of all of these phytochemicals indicates that plants have great potential to yield GMs to manage several diseases, including diabetes mellitus. The use of the plant has been mentioned in the folklore inventory of wild plants from AJK, Pakistan, a study (Ishtiaq et al. 2021) Where the traditional use of GM from the plant under study has been illustrated in managing diabetes and skin diseases.

The blood glucose levels of the model animals significantly decreased upon administration of *L. longiflorus* medicaments (Figure 1). This decrease in glucose levels might be due to the alkaloids present in the extract of the plant. Higher alkaloid levels lead to lower glucose synthesis from liver cells. Furthermore, the capacity of pancreatic β-cells increases, and more insulin production occurs due to the presence of alkaloids in the extract (Kamran et al. 2024). Alkaloids activate AMP-activated protein kinase (AMPK), leading to improved insulin sensitivity. Furthermore, flavonoids also exhibit antioxidant and anti-inflammatory properties, which can contribute to overall metabolic health. Triterpenoids have demonstrated anti-diabetic effects by enhancing insulin sensitivity and promoting glucose uptake (Mushtaq et al. 2016). They may also have protective effects on pancreatic beta cells. Soluble fiber of the methanolic extracts, in particular, can slow the absorption of glucose, helping to regulate blood sugar levels. Overall, it can be inferred that leaf extracts from *L. longiflorus* may serve as potential candidates for ameliorating higher blood glucose levels and that these plants should be used in the synthesis of GM.

The body weights of the rabbits were analyzed in the current study (Figure 2), as an increased body weight significantly correlates with insulin resistance. The greater the weight of an organism the more resistant it will be to insulin (Mushtaq et al. 2016). A reduction in the body weight of rabbits leads to improved insulin action, which ultimately reduces elevated blood sugar levels. The application of *L. longiflorus* medicament resulted in the normalization of body weights in the rabbits. The plant extracts may influence metabolic pathways (Bharti et al. 2018), promoting the breakdown of fats and increasing energy expenditure. This can contribute to weight loss by reducing the accumulation of excess fat. Furthermore, plant compounds can influence hormone levels, including those related to metabolism and insulin sensitivity. By modulating hormone activity, these extracts may contribute to weight management and improved insulin function (Efenberger-Szmechtyk, Nowak, and Czyzowska 2021). A better phytochemical profile of *L. longiflorus* from the host *A. lebbeck* might be a reason for better bioactivity.

The serum insulin profile increased following the administration of *L. longiflorus* medicament (Figure 3). The serum insulin concentrations are associated with health and the number of pancreatic beta cells involved in endogenous insulin production (Kundo et al. 2021). Plant extracts rich in antioxidants and anti-inflammatory compounds may help protect pancreatic beta cells from oxidative stress and inflammation, promoting their health and function. For instance, polyphenols are better known as serum insulin boosters. Polyphenols are a large group of naturally occurring compounds found in plants. They have antioxidant properties and are known for their potential health benefits (Kristiani and Kasmiyati 2021). Studies have reported the occurrence of polyphenols in the phytochemical profile of mistletoes (Ariani et al.

2023, Mushtaq et al. 2023). Additionally, plant compounds have been investigated for their potential to promote the regeneration or proliferation of pancreatic beta cells, which could contribute to increased insulin production. The phytochemical profile of the *L. longiflorus* revealed the presence of flavonoids and tannins, which are polyphenolic compounds. It is plausible that these compounds might have boosted serum insulin levels of the diabetic rabbit models due to their potential antioxidant role (Ariani et al. 2023, Mushtaq et al. 2023).

5. Conclusion

In conclusion, this study demonstrates the noteworthy antidiabetic potential of *Loranthus longiflorus* sourced from two distinct host species. Employing the EPA model, the phytochemical profile of the plant was determined using standardized protocols. The findings reveal the abundance of phytochemicals in *L. longiflorus*, positioning it as a promising candidate for GM. The evaluation of antidiabetic potential involved scrutinizing blood glucose levels, serum insulin levels, and body weights in rabbits. Notably, the 1000 mg dose of methanolic extract from the leaf of *L. longiflorus* from the host *A. lebbeck* exhibited remarkable antidiabetic activity, synergistically lowering blood glucose levels and body weight in rabbits, while concurrently elevating serum insulin levels. This study underscores *Loranthus longiflorus* as a compelling contender for GM, emphasizing its robust anti-hyperglycaemic efficacy. For future research, exploring the molecular mechanisms behind these effects, conducting clinical trials in human subjects, and assessing long-term safety and efficacy would be valuable avenues to further validate the therapeutic potential of *L. longiflorus* in diabetes management. Additionally, investigating optimal dosages and potential synergies with conventional treatments could enhance its clinical applicability.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability

All the data related to this manuscript is available with the authors.

Study Approval

This study was approved by the Departmental Ethics Committee (DEC), Mirpur University of Science and Technology, Mirpur, Pakistan for the Care of Animals.

Consent Forms

All participants provided written informed consent, available with the authors.

Author Contributions

All authors contributed to the study's conception and design. MM and MZA performed the experiments, MI performed data collection and analysis, and the first draft was written by MWM. Review and editing were performed by MM, MWM, and MI. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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