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Research

COMPARATIVE *In-Vitro* ANTIDIABETIC EVALUATION OF ETHANOLIC AND CHLOROFORM *ALBIZIA AMARA* LEAF EXTRACTS

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Abstract

Background: Diabetes is a major global health concern, impacting more than 540 million persons globally. Type 2 diabetes mellitus is the most common variety, and it is mostly associated with poor eating habits, sedentary lifestyles, and rising obesity rates, particularly in developing nations. The rising prevalence of this metabolic condition raises serious health concerns and highlights the urgent need for safer and more effective antidiabetic medications. In recent years, plants used in traditional medicine have received interest as possible sources of new antidiabetic chemicals. *Albizia amara*, a plant often used in herbal medicine, has been shown to contain a number of bioactive components that may contribute to its therapeutic effects.

Aim: The present research sought to assess and compare the antidiabetic effects of ethanolic and chloroform leaf extracts of *Albizia amara*. The study also looked at how solvent polarity affected phytochemical extraction and biological activity.

Method: The phytochemical content of both extracts was investigated using qualitative tests to identify important bioactive chemicals such as flavonoids, alkaloids, tannins, glycosides, and saponins. The antidiabetic potential of the extracts was then investigated using a typical experimental diabetes model, with voglibose serving as the reference medication. Blood glucose levels were measured to determine the effectiveness of each extract in managing hyperglycemia.

Result: Phytochemical screening revealed the presence of many bioactive chemicals in both extracts, indicating potential therapeutic value. The ethanolic extract reduced blood glucose levels more than the chloroform extract, implying that higher-polarity solvents may improve the extraction of active components responsible for antidiabetic actions.

Conclusion: The data show that *Albiziaamara* has strong antidiabetic

potential, which supports its traditional use in diabetes management. The greater efficacy of the ethanolic extract emphasizes the significance of solvent choice in phytochemical extraction. Further research into chemical isolation, characterization, and mechanistic investigations is needed to fully understand *Albiziaamara's*medicinal potential and involvement in diabetes treatment.

Keywords: Diabetes mellitus, *Albiziaamara*, antidiabetic activity, phytochemicals, herbal medicine.

INTRODUCTION

Herbal medicine, also known as phytotherapy the use of plants and their extracts for therapeutic purposes forms the cornerstone of many traditional healthcare systems that have been practiced for centuries.[1] These systems emphasize holistic health and aim to maintain the body's balance rather than treating isolated symptoms. In India, herbal medicine is deeply integrated into traditional disciplines, including Ayurveda, Siddha, Unani, Yoga, Naturopathy, and Homeopathy, which rely extensively on natural formulations to prevent and manage diseases.[2] Unlike synthetic drugs that usually act on specific biochemical pathways, herbal preparations often exert multifaceted effects such as antioxidant, anti-inflammatory, and metabolic regulatory actions, thereby contributing to overall well-being.[3]

Globally, herbal medicine has gained renewed interest because of the rising incidence of chronic diseases like diabetes, hypertension, obesity, and cardiovascular disorders, coupled with the increasing cost and side effects associated with synthetic medications. Additionally, there has been a shift in public preference towards natural, safe, and sustainable healthcare alternatives. The global herbal medicine market, valued at approximately USD 148.5 billion in 2022, is projected to reach around USD 386.1 billion by 2032, growing at a compound annual rate of about 11.2% .[4,5] According to another estimate, the market for medicinal plants and their products is expected to rise from USD 14 billion to nearly USD 5 trillion by 2050 (Global Research Online, 2019). This global trend highlights not only the economic significance of herbal remedies but also the increasing recognition of their therapeutic potential and the need for scientific validation of traditional knowledge.[5,6]

Diabetes mellitus is one of the most serious metabolic disorders of modern times, characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both [5]. It is classified mainly into four categories: Type 1 diabetes mellitus (T1DM), caused by autoimmune destruction of pancreatic β-cells; Type 2 diabetes mellitus (T2DM), which accounts for about 90–95% of all diabetes cases and results from insulin resistance coupled with β-cell dysfunction; gestational diabetes mellitus (GDM), which occurs during pregnancy; and secondary forms caused by pancreatic diseases, genetic abnormalities, or drug-induced conditions.[7,8] The increasing prevalence of diabetes globally poses a major public health challenge, leading to complications such as neuropathy, nephropathy, retinopathy, cardiovascular diseases, and delayed wound healing.[9,10]

Conventional pharmacological treatment for diabetes includes several drug classes, such as α-glucosidase inhibitors, DPP-4 inhibitors, GLP-1 receptor agonists, thiazolidinediones, biguanides, sulfonylureas, meglitinides, insulin and its analogues, and SGLT-2 inhibitors.[11] Although these medications are effective in maintaining glycemic control, their long-term use is often associated with adverse effects, including hypoglycemia, lactic acidosis, gastrointestinal discomfort, pancreatitis, weight gain, and cardiovascular complications. Furthermore, the progressive nature of diabetes often reduces the efficacy of these drugs over time, leading to the need for combination therapies or insulin dependence.[9,12,13] Hence, there is growing scientific interest in identifying natural alternatives that can control hyperglycemia with fewer side effects while offering additional health benefits.

One of the key therapeutic strategies in managing diabetes involves the inhibition of carbohydrate-hydrolyzing enzymes such as α-glucosidase and α-amylase, which are responsible for the breakdown of complex carbohydrates into simple sugars in the small intestine. Inhibition of these enzymes slows down glucose absorption, thereby reducing postprandial hyperglycemia. The potency of enzyme inhibitors is measured using the half-maximal inhibitory concentration (IC_{50}) value, where a lower IC_{50} indicates higher inhibitory activity.[14,15] Natural inhibitors

derived from plants, especially those rich in flavonoids, tannins, and phenolic acids have been shown to exhibit strong α-glucosidase inhibitory effects comparable to synthetic drugs like acarbose and voglibose.[16] Thus, investigating plant extracts for their enzyme-inhibitory activity is an important approach in developing novel antidiabetic therapies.[17]

Albizia amara (Fabaceae), a deciduous tree native to tropical and subtropical regions of Africa and India, has been widely used in traditional medicine for the treatment of various ailments. In Indian traditional systems, different parts of the plant, including leaves, bark, flowers, seeds, and wood, are used to treat conditions such as gonorrhea, skin diseases, leprosy, diarrhea, cough, and poisonous bites.[18] Phytochemical screening of *Albiziaamara* has revealed the presence of several bioactive compounds, including flavonoids, alkaloids, terpenoids, glycosides, phenols, tannins, saponins, and steroids. These compounds are known for their pharmacological activities, such as antioxidant, antimicrobial, hepatoprotective, and antidiabetic effects.[19] The pharmacological potential of*Albizia amara* has been supported by a number of studies, which reported that the bark extract of *Albiziaamara* exhibited significant antihyperlipidemic activity in Triton X-100-induced hyperlipidemic rats, suggesting its possible role in improving lipid metabolism.[20] Similarly, other species within the same genus, such as *Albizia lebbeck* and *Albizia odoratissima*, have shown potent α-glucosidase inhibitory activity *in-vitro*, with IC_{50} values comparable to standard antidiabetic drugs. These findings support the hypothesis that *Albizia amara* may possess similar enzyme inhibitory properties, making it a promising candidate for further antidiabetic research.[21]

Given the limitations of conventional therapies and the therapeutic potential of medicinal plants, the goal of the current study is to evaluate *Albizia amara's* antidiabetic potential. This entails collecting and classifying the plant, preparing various extracts, qualitatively screening for phytochemicals, and evaluating the hypoglycemic effect in vitro. The study aims to support its traditional use with scientific evidence and explore its potential as a natural source of antidiabetic compounds.

MATERIALS AND METHOD

In December 2024, fresh leaves of *Albizia amara* (Fabaceae) were gathered from Naikanpatty in Tamil Nadu, India's Salem District, and verified before being used. A mechanical grinder was used to grind the gathered leaves into a coarse powder after they had been properly cleaned with distilled water to get rid of dust and dirt and shade-dried after a short period of exposure to the sun. To guarantee consistent particle size, the powdered leaves were run through sieve No. 25 and kept in sealed containers until additional examination.

Petroleum ether, chloroform, and ethanol were the solvents of increasing polarity used in a Soxhlet apparatus to extract the powdered leaf material successively. Each solvent was used to extract about 148 g of powdered material over the course of 48 hours. A rotary evaporator was used to concentrate the resultant filtrates at lower pressure, and the solid wastes were stored in desiccators for use in further tests.[21]

Using the usual procedures outlined, the powdered leaves and the subsequent extracts were subjected to qualitative phytochemical screening in order to identify the primary groups of secondary metabolites. By observing the creation of turbidity or precipitate, the Mayer, Dragendorff, Wagner, and Hager tests verified the presence of alkaloids. While carbohydrates were verified by Molisch's, Fehling's, and Benedict's tests, which produced violet or brick-red color production, amino acids was identified using Ninhydrin and Millon's tests, which produced purple or red coloration. The Shinoda and Alkaline reagent tests were used to identify flavonoids by looking for yellow or pink coloring. Ferric chloride and lead acetate tests confirmed the presence of tannins and phenolic compounds, yielding blue-black or white precipitates, whereas Keller–Killiani and Borntrager's tests identified glycosides, giving blue-green or pink colors. Using the Biuret and Xanthoproteic assays, proteins were recognized by their respective violet or yellow coloring. Using the Salkowski and Liebermann–Burchard tests, steroids and triterpenoids were identified by the development of red, green, or blue colors. The presence of a number of bioactive components, including alkaloids, flavonoids, glycosides, tannins, phenolics, saponins, proteins, and steroids, was determined by the qualitative analysis. These components are known to support the pharmacological and possible antidiabetic effects of the plant.[23]

The method outlined by Apostolidis was used to determine the *Albizia amara* ethanol (EHEE) and chloroform (EHCE) extracts' α-glucosidase inhibitory activity. The assay measured the 4-nitrophenol release from p-nitrophenyl

glucopyranoside (pNPG) at 405 nm using a UV–VIS spectrophotometer. At pH 6.8, 100 µM phosphate buffers were used to create the substrate solution (pNPG). 200 µL of α-glucosidase enzyme solution was pre-incubated for 10 minutes at 37°C with different concentrations of the plant extracts (10, 20, 40, 80, 160, and 320 µg/mL) in order to prepare for the experiment.400 µL of a 5.0 mM pNPG solution made in the same buffer was added to start the reaction, which was then incubated for 20 minutes at 37°C. 1 mL of 0.1 M sodium carbonate (Na_2CO_3), which turned yellow from the production of 4-nitrophenol, was added to stop the process. Using voglibose as a positive reference, the absorbance of the resultant combination was measured at 405 nm using a UV–VIS spectrophotometer.[24]

Using the following formula, the % inhibition of α-glucosidase activity was determined:

% Inhibition = [(Abs Control - Abs Sample) / Abs Control] x 100

RESULT AND DISCUSSION

Albizia Amara's powdered leaves, which are a member of the Fabaceae family, have been studied methodically, starting with sequential extraction. Antidiabetic efficacy was tested in a preliminary phytochemical analysis to determine the active ingredient in the plant's chloroform and ethanolic leaf extracts. For the current experiment, the leaves of *Albiziaamara*were chosen based on ethanobotanical data that indicates their antibacterial, anticancer, antioxidant, antimalarial, and anti-inflammatory properties. In the Salem district, close to Naikanpatty, the plant material was gathered. Pet ether, chloroform, and ethanol were used to extract the powdered leaves using Soxhlet equipment. At a regulated temperature and with less pressure, the extract was dried out.

PRELIMINARY PHYTOCHEMICAL STUDIES:

Preliminary investigations into the phytochemistry of*Albizia amara* leaves. Initial phytochemical analyses of *Albizia amara* leaf extracts show that the chloroform and ethanol extracts contain proteins, alkaloids, flavonoids, and tannins.

The plant's phytochemical constituent was displayed in table 1 and the fig 1,2,3,4 shows the visual representation of all the tests.

Table 1: Qualitative Phytochemical Screening of *Albiziaamara* Extracts:

Name of the chemical constituent	Test	Acetone	Ethanol	Water	Petroleum ether
Alkaloids	a) Mayers Test	+	+	+	-
	b)Dragendro's Reagents	+	+	+	+
Glycosides	Borntrager's Test	-	-	+	-
Steroids	LibermanBurchard's	-	+	+	-
Terpenoids	Salkowski Test	-	+	+	-
Flavonoids	a) HCL	-	+	+	-
	b) Sodium Hydroxide	-	-	+	-

Phenols	Ferric chloride Test	+	+	+	-
Saponins	Sodium Bicarbonate	-	-	+	-

(+) = Indicates presence (-) = Indicates absence

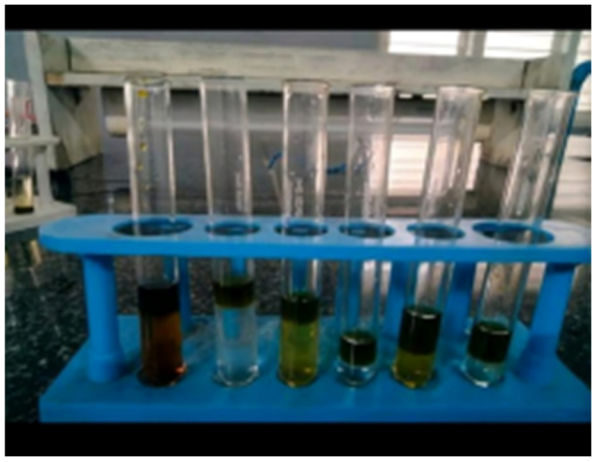

Figure 1: Preliminary Phytochemical Screening with Petroleum

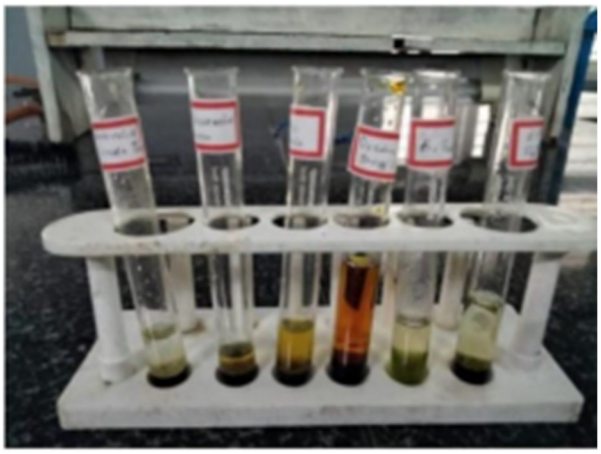

Figure 2: Preliminary Phytochemical Screening With Water

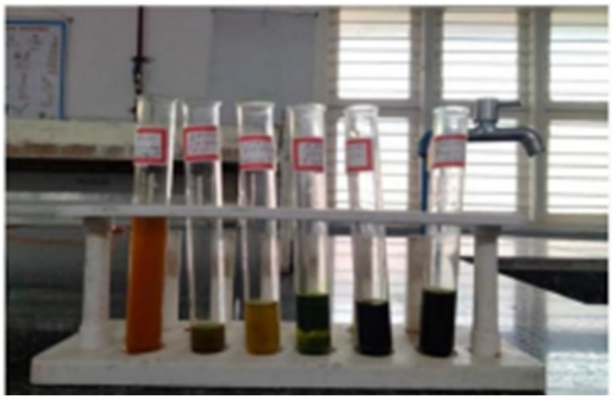

Figure 3: Preliminary Phytochemical Screening with Acetone

Figure 4: Preliminary Phytochemical Screening with Methanol

IN VITRO ANTIDIABETIC ACTIVITY:
Herbal medications are an economical and efficient option to treat type 2 diabetes since they suppress key enzymes associated with the disease. Comparing the α-glucosidase inhibitory activities of the chloroform and ethanolic extracts to the positive control, voglibose, revealed a notable inhibitory effect against the enzyme.
To ascertain the α-glucosidase inhibitory activity of chloroform and ethanol extract of Albiziaamara, spectrophotometry was used at various sample concentrations (10, 20, 40, 80, 160, and 320). Voglibose was used as a guide. They measured the absorption.

ALPHA GLUCOSIDASE INHIBITION ASSAY:
The sample extracts (AAC and AAE) were tested for α-glucosidase inhibitory activity and compared to the standard medication, voglibose. Table 2 shows the % inhibition at different concentrations (10-320 µg/mL), the fig 5, 6, 7 shows the visual representation of all the assay test.

Table 2: Alpha glucosidase inhibitory activity of standard (Voglibose) and samples (AAC and AAE).

Conc.(µg)	VOGLIBOSE	AAC	AAE
10	14.55117657	9.907184051	11.29990206
20	55.52023293	37.92498142	39.47297893
40	74.35452244	45.14992863	52.88991617

80	84.10578798	53.76695187	59.95894191
160	91.12417561	68.72965207	70.27764958
320	94.37608815	75.49083806	83.2308256

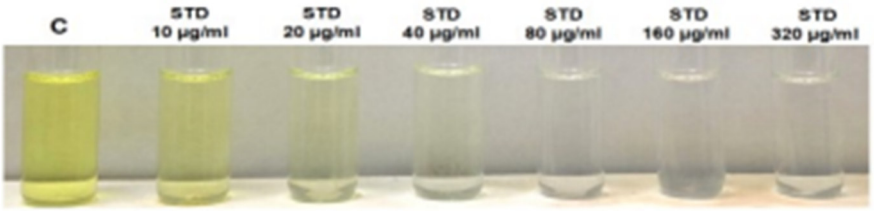

Figure 5: Alpha glucosidase inhibition assay on control and Standard

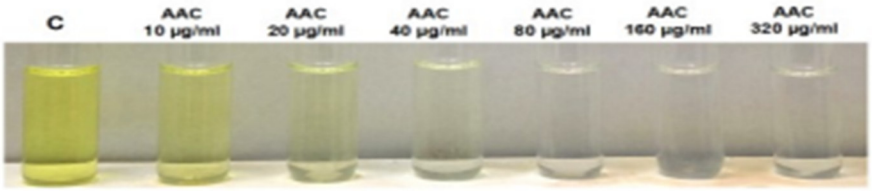

Figure 6: Alpha glucosidase inhibition assay on chloroform Extract

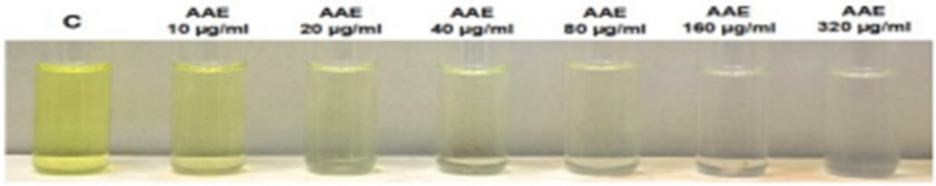

Figure 7: Alpha glucosidase inhibition assay on ethanolic extract

The study found that AAC and AAE inhibited the α-glucosidase enzyme in a dose-dependent manner, with

higher concentrations leading to greater inhibition. AAE outperformed AAC in terms of inhibitory action at all concentrations tested, albeit both remained lower than the standard voglibose. AAE may play a function in reducing postprandial hyperglycemia due to its ability to block α-glucosidase more effectively

α- GLUCOSIDASE INHIBITION ASSAY:

The impact of the samples (AAC, AAE) on α-glucosidase activity was assessed using the α-glucosidase enzyme and the methodology outlined by Apostolidis [1]. Using 100 mM phosphate buffer and a pH of 6.8, the substrate solution p-nitrophenyl glucopyranoside(pNPG) was made. Various extract concentrations (10, 20, 40, 80, 160, and 320) were pre-incubated with 200 µL of α-glucosidase for 10 minutes. To initiate the reaction, 400 µL of 5.0 mM (pNPG) substrate dissolved in 100 mM phosphate buffer (pH 6.8) was then added. One milliliter of Na_2CO_3 (0.1 M) was added to the reaction mixture to stop it after 20 minutes of incubation at 37 °C. A UV-VIS spectrophotometer was used to quantify the 4-nitrophenol reaction mixture, which was yellow in color and released from pNPG, at 405 nm. The following formula was used to determine the inhibitory activity of α-glucosidase, with voglibose serving as a positive control. [(Abs Control - Abs Sample) / Abs Control] x 100 = % Inhibition The standard medication, Voglibose, and the provided samples, AAC and AAE, have IC50 values of 48.64 µg/mL, 61.59 µg/mL, and 23.13 µg/mL, respectively.

CONCLUSION

Herbal medications are highly valued for their intrinsic properties, such as cost-effectiveness and few side effects, making them an excellent alternative for treating chronic conditions. Despite the availability of many synthetic medications, plant-based remedies remain popular due to their perceived safety and efficacy. Diabetes remains one of the leading risk factors for developing health complications that interfere with everyday life, emphasizing the importance of effective and safe antidiabetic treatments. The leaves of *Albizia amara*, a member of the Fabaceae family, were studied for their phytochemical composition and antidiabetic potential. The chloroform and ethanolic extracts were phytochemically analyzed and found to include alkaloids, amino acids, volatile and fixed oils, flavonoids, glycosides, mucilage, tannins, proteins, and steroids, confirming the plant's therapeutic potential. The extracts' antidiabetic efficacy was assessed *in-vitro* using the α-glucosidase inhibition assay. Both the ethanolic and chloroform extracts had strong dose-dependent antidiabetic effects. The IC50 values for the ethanolic extract, chloroform extract, and standard medication voglibose were 48.64 µg/ml, 61.59 µg/ml, and 23.13 µg/ml, respectively, showing significant enzyme inhibition. Interestingly, in the in vitro model, the chloroform extract showed slightly greater activity than the ethanolic extract. These data indicate that *Albizia amara* leaves have significant antidiabetic effects, which supports their traditional use in diabetes management. Additional research, including chemical isolation, mechanistic studies, and clinical trials, could open up new avenues for incorporating this plant into modern therapeutic methods to diabetes therapy.

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