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SHORT COMMUNICATION

Phytochemical Screening and α-Glucosidase Inhibitor Activity of Aerial Parts of Maidenhair Fern: *Adiantum lunulatum*

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Abstract

Background: Medicinal plants and herbs are used extensively in traditional medicine to treat and manage a wide range of conditions, including diabetes mellitus and its after-effects. As diabetes mellitus is increasing rapidly and is mostly caused by changes in lifestyle, the use of traditional medicines for its treatment and management has increased. Fern extracts have demonstrated tremendous potential in the management of diabetes, cancer, cardiovascular problems and neurological disorders. A prominent class of type 2 antidiabetic drugs and α-glucosidase inhibitors work by lowering postprandial hyperglycemia. **Aim:** The purpose of this *in vitro* investigation was to assess any possible inhibitory effects of aerial portions of the Maidenhair Fern (*Adiantum lunulatum* Burm). **Methods:** Using extracts of *Adiantum lunulatum* derived from methanolic and chloroform solvents, the research team performed phytochemical screenings to ascertain if the extracts could stop α-glucosidase. **Results:** Minimising the postprandial plasma glucose spikes is achieved by delaying the absorption of carbs and glucose by inhibiting the activity of α-glucosidase. The chloroform extract of *Adiantum lunulatum* exhibited an IC_{50} value of 705.02 ± 2.122µg/ml and the methanol extract exhibited an IC_{50} value of 526.12 ± 1.155 µg/ml. Acarbose was used as a positive control with an IC_{50} value of 314.11 ± 1.021µg/ml. The methanol extract exhibited more potent alpha-glucosidase inhibitory activity than other extracts. The phytochemical screening of the extracts showed the presence of polyphenols and flavonoids. The microscopic study of the rachis of the fronds was carried out which showed the presence of a single layer of epidermic, sclerenchyma, stele and exarch xylem which helps in authentication. **Conclusion:** These findings can pave the way for the development of novel medicinal compounds derived from the Maidenhair Fern and bring more standardisation to the use of traditional herbal therapies for the treatment of diabetes mellitus.

Keywords: Acarbose, *Adiantum lunulatum*, α-glucosidase Inhibitor Activity, Maidenhair Fern, Type 2 Diabetes

1. Introduction

Diabetes Mellitus (DM) is a metabolic condition characterised by an inadequate utilisation of insulin, hence impeding the body's capacity to metabolise carbohydrates.

A decrease in insulin production due to the absence of functioning beta cells causes type I diabetes, which is insulin dependent. Type I diabetics must rely on an external insulin supply for all of their insulin needs, while Type II diabetics, who are insulin-independent, can manage their condition with lifestyle modifications including eating better and exercising more, as well as with medicine. Type II diabetes accounts for 90% of all cases of diabetes, making it the most frequent of the two types. High blood sugar, unusual thirst, frequent urine, severe hunger, blurred vision, nausea, vomiting, extreme weakness, extreme fatigue, irritability, mood swings and other symptoms can be seen in both types of diabetes[1].

Damage to and potential failure of organ functions are long-term effects of DM. Worldwide, the incidence of diabetes mellitus has been on the rise in recent decades, according to the World Health Organization (WHO). From an anticipated 4% in 1995 to 5.4% in 2025, the worldwide prevalence of diabetes is projected to rise.

A resurgence of curiosity about the potential of medicinal plants as a diabetic treatment has recently

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emerged. Many commonly used medications have their origins in medicinal plants and their prototypic molecules. Because plants contain numerous bioactive phytochemical components with diverse beneficial biological effects, using decoctions over the course of treating complicated disorders like DM has the potential to provide an effective and inexpensive multi-targeted therapeutic approach. Oxidative stress, caused by the mitochondria's overproduction of free radicals, has been associated with DM[2].

Phytochemicals, such as flavonoids and polyphenols can help cure DM by reducing oxidative stress and free radicals. They prevent high blood sugar levels by attaching to glucose transporters and blocking the digesting enzymes (α-amylase and α-glucosidase) by competitive inhibition. Terpenes, alkaloids and saponins are some of the secondary plant metabolites that may control glucose absorption and utilisation while also increasing insulin release[3].

One of the major risk factors for developing type 2 diabetes and its consequences is postprandial hyperglycemia[4,5]. Inhibiting the enzymes that hydrolyse carbohydrates such as α-amylase and α-glucosidase[6] is one way to slow down the increase of blood glucose levels after eating. Inhibiting alpha-glucosidase is a useful tool for controlling postprandial glucose levels. Reduced postprandial plasma glucose rise is the result of a slower rate of glucose absorption brought about by the inhibition of certain enzymes, which postpone or even stop the breakdown of carbohydrates[7,8].

Hypoglycemia, diarrhoea, gas and intestinal bloating are some of the side effects of the current clinically used alpha-amylase and glucosidase inhibitors, which limit their capacity to treat diabetes and its complications[9].

A member of the Pteridophyte family, ferns have been around for millions of years and are found all over the globe. Ferns are understudied in comparison to other plant genera, despite their widespread human use for medical and industrial uses. Numerous investigations have revealed the presence of potent pharmacological effects in secondary metabolites found in ferns, providing insight into the intricate biochemistry of these plants[10].

Furthermore, fern extracts have shown promise in the treatment of several illnesses, including diabetes, cancer, cardiovascular issues and neurological disorders, according to a study[11,12].

"Hanspadi" and "Walking Maidenhair Fern" are two common names for the *Adiantum lunulatum* Burm F. species. It is a popular decorative plant with a broad distribution across India[13]. It has an ethnomedical use for conditions such as elephantiasis, epilepsy, diarrhoea, burning sensation, erysipelas and epilepsy[14,15]. The whole dried plant has a history of use as a cough and bronchitis remedy. There have been reports of *A. lunulatum* Burm F having anti-bacterial and anti-fungal properties as well[16,17]. Isolated and discovered phyto-constituents from various plant parts include carotenoids, flavonoids, nortriterpeneadiantone and others[18-21]. Conditions like erysipelas, burning sensation, epilepsy, diarrhoea, strangury and elephantiasis can benefit from its use. Yet, there has been scant evidence to support its pharmacological actions, including antioxidant and hypotensive effects.

2. Materials and Methods

2.1 Acquisition of Plant Materials

Aerial parts of *Adiantum lunulatum* Burm. f. were collected from Seshachalam hills, Tirupati and authenticated by Dr V. Rama Rao, Central Ayurveda Research Institute, Uttarhalli, Bengaluru and the number on the voucher was RRCBI-4546.

2.2 Preparation of Extracts

Plant material was air-dried by layering it on top of clean polythene sheets in a shaded area with good ventilation. The plant material was turned over often to promote drying and prevent the formation of microbes. After drying, the material was sieved through mesh no.80 and ground into a coarse powder. Approximately 500 grams of *A. lunulatum* powder was soxheleted with hexane for 24 hours followed by chloroform and methanol solvent extraction for 6 hours each. A rotary evaporator was used to collect the supernatant and evaporate it using reduced pressure until it was completely dry. After that, the weight of the extracted substances was determined and the yield was computed. The well-sealed, labelled containers were used to keep the dried extracts for future research.

2.3 Determination of Physicochemical Parameters

Based on the techniques outlined in the Indian Pharmacopoeia standard, physicochemical

parameters such as the value of ash[22] and extractive[23], foreign organic matter[24] and loss when drying[25] out were established. Inorganic residues are represented by the ash value, while the amount of active components present can be confirmed by the extractive value, which depends on the solvent used. Moisture content and volatile principle measurements allow one to ascertain loss on drying. Any organ or tissue from a crude medicine not specifically mentioned in the drug's description or definition is considered foreign organic matter. The quality of the raw material can be better understood by determining all of these parameters.

2.4 Microscopic Study

The fronds were washed, the transverse section of the rachis was consumed and choral hydrate was cleared to observe the anatomy with the help of a Quasmo binocular compound microscope[26].

2.5 Preliminary Phytochemical Screening of the Extracts

Chloroform and methanolic extracts from *A. lunulatum* were subjected to preliminary screening of the plant constituents. Standard protocols were followed to examine the extracts in light of the various main and secondary metabolites present[27].

2.6 Alpha-glucosidase Inhibitory Activity

We used the conventional approach with a few tweaks to measure the extracts' alpha-glucosidase inhibitory activity[28]. At 37°C, a 96-well plate was preincubated for 15 minutes with a reaction mixture that included 10µL of alpha-glucosidase (IU/ml), 50µL of phosphate buffer (100mM, pH = 6.8), and 20µL of extract and fractions at various concentrations (0.1, 0.2, 0.3, 0.4, and 0.5mg/ml). The following step involved adding 20µL of the substrate P-NPG (5mM) and continuing to incubate at 37°C for another 20 minutes. Fifty microliters of sodium carbonate (0.1 M) were added to halt the reaction. We measured the absorbance of the released p-nitrophenol at 405nm using a Multiplate Reader. Acarbose was added as a control at concentrations between 0.1 and 0.5mg/ml. As a control, an experiment was conducted in triplicate without the test material. The percentage inhibition was used to express the results, which were derived using the formula,

$$\text{Inhibitory activity (\%)} = \frac{1 - A_s}{A_c} \times 100$$

Where, - the absorbance when the test material is present and - the control's absorbance.

3. Results

A. lunulatum Burm. F. is a kind of fern from the Pteridaceae family. Known by most as the "Walking Maidenhair fern," *Hamsapadi* is a beautiful plant. *A. lunulatum* has been used medicinally by several cultures in India to treat conditions like diarrhoea, ulcers, burning sensations, chest pain and snake bites[29]. Pedanius Dioscorides mentions that it has been used as a medicine from ancient times. It acts as an anti-dysenteric, antiulcer, antimicrobial, antitumour and antiviral and is used as an astringent, demulcent, diuretic, emmenagogue, expectorant and tonic[30]. Given the above mentioned wide pharmacological actions, an attempt was made to explore the less studied anti-diabetic activity of the plant's aerial portions using the α-glucosidase inhibitory assay.

3.1 Determination of Physicochemical Parameters

Physicochemical parameters of the extracts like ash values, extractive values, moisture content and foreign organic matter were analysed according to the standard pharmacopeial methods and results were tabulated in Table 1.

Table 1. Physico-chemical analysis of powder of aerial parts of *A. lunulatum*

S. No.	Parameter	% w/w
Ash Values		
1	Total ash	9.8 ± 0.2
2	Acid insoluble ash	4.7 ± 0.2
3	Water soluble ash	2.1 ± 0.0
Extractive Values		
1	Water soluble extractive	5.6 ± 0.2
2	Ethanol soluble extractive	3.3 ± 0.2
Loss on drying		
		9.3 ± 0.3
Foreign Organic Matter		
		1.7 ± 0.1

Each value (n = 3) reflects the mean ± SEm.

3.2 Microscopic Study

An adaxial groove and an abaxial convex shape were visible in the rachis's Transfer Section (TS). The single-layered epidermis was heavily cutinised. The hypodermis is composed of parenchymatous ground tissue and two to four layers of sclerenchyma. Near the notch, which looked like a plate, was a monostele. The protostele was mostly made up of a xylem, with a protoxylem at either end and a metaxylem in the middle. This revealed an exarch condition that was filled in by a ceratenchyma, which consisted of fewer phloem cells. The cells in the notch area showed minimal stretching (Figure 1).

3.3 Preliminary Phytochemical Screening of the Extracts

The findings of the initial phytochemical screening of the methanol and chloroform extracts of the plant's aerial portions are shown in Table 2. Alkaloids, glycosides, tannins, flavonoids, and saponins were found via phytochemical analysis.

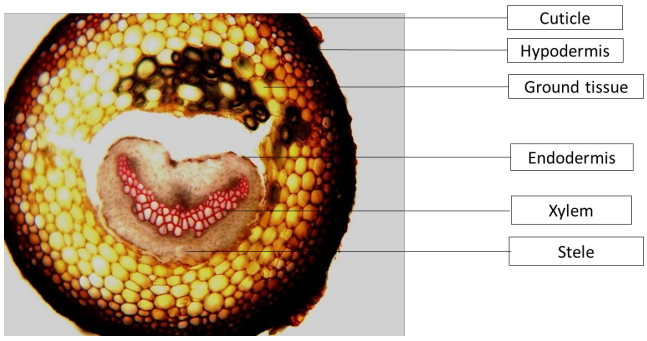

Figure 1. TS of aerial pars of *Adiantum lunulatum* plant.

Table 2. Phytochemical screening of chloroform and methanol extracts of aerial parts of *A. lunulatum*

Constituents	Chloroform Extract	Methanol Extract
Carbohydrates	-	-
Alkaloids	+	+
Glycosides	-	+
Steroids	-	-
Saponins	+	-
Tannins	+	+
Flavonoids	+	+
Proteins and amino acids	-	-

(+): present; (–): not detected.

3.4 Alpha-glucosidase Inhibitory Activity

Alpha-glucosidase inhibition assay was used to examine the antidiabetic effects of aerial component extracts *in vitro* using chloroform and methanol. Acarbose, a typical medication that specifically inhibits α glucosidase, was compared to the extracts' inhibitory efficacy. Different concentrations ranging from 50 to 800µg/ml of chloroform, methanol and acarbose were screened for *in vitro* α-glucosidase inhibitory activity. Comparing the methanol extract to the chloroform extract, the former showed better inhibitory action. Acarbose exhibited the highest percentage of inhibition of 72.56 ± 1.22 at a concentration of 800µg/ml. The percentage inhibition of chloroform extract was found to be 53.21 ± 0.22 and 61.07 ± 0.34 for methanol extract at a concentration of 800µg/ml. The IC_{50} value of chloroform, methanol and acarbose was found to be 705.02 ± 2.122, 526.12 ± 1.155 and 314.11 ± 1.021 respectively. The result of the percentage inhibition and Table 3 contains the IC50 values for the extracts and standard acarbose, along with accompanying plots (Figures 2, 3, 4 and 5). From these findings, it can be

Table 3. Alpha-glucosidase-inhibiting properties of *A. lunulatum* chloroform and methanol extracts

Sample	Concentration (µg/ml)	% Inhibition	IC 50 µg/ml
Methanolic Extract *Adiantum lunulatum*	50	15.59 ± 0.13	526.12 ± 1.155
	100	28.83 ± 0.14	
	200	36.48 ± 0.34	
	400	49.24 ± 0.35	
	800	61.07 ± 0.34	
Chloroform Extract *Adiantum lunulatum*	50	7.69 ± 0.11	705.02 ± 2.122
	100	14.65 ± 0.27	
	200	22.45 ± 0.62	
	400	36.20 ± 0.46	
	800	53.21 ± 0.22	
Acarbose (Standard)	50	22.77 ± 0.21	314.11 ± 1.021
	100	40.73 ± 1.39	
	200	49.34 ± 1.04	
	400	63.48 ± 0.91	
	800	72.56 ± 1.22	

The value represents Mean ± SEm (n=3)

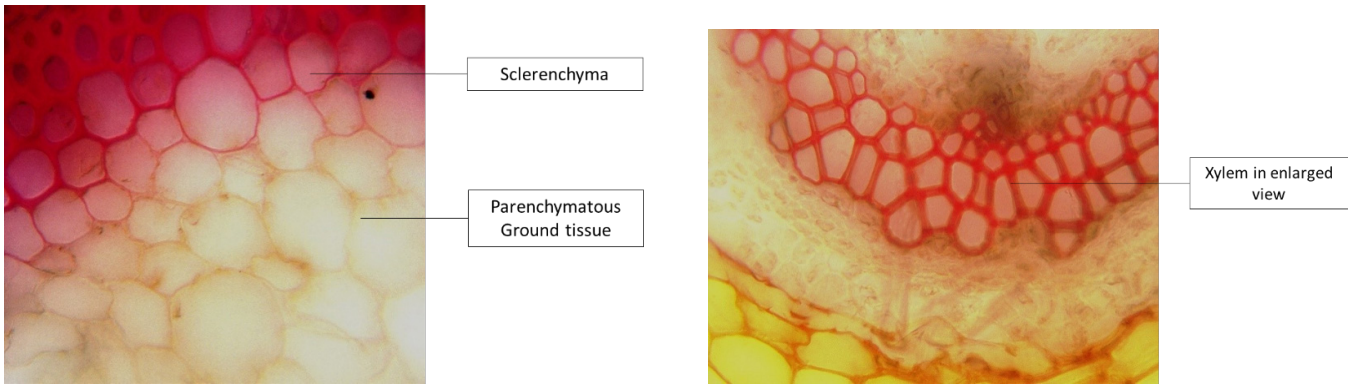

Figure 2. Alpha-glucosidase inhibitory activity of chloroform extract of *Adiantum lunulatum*.

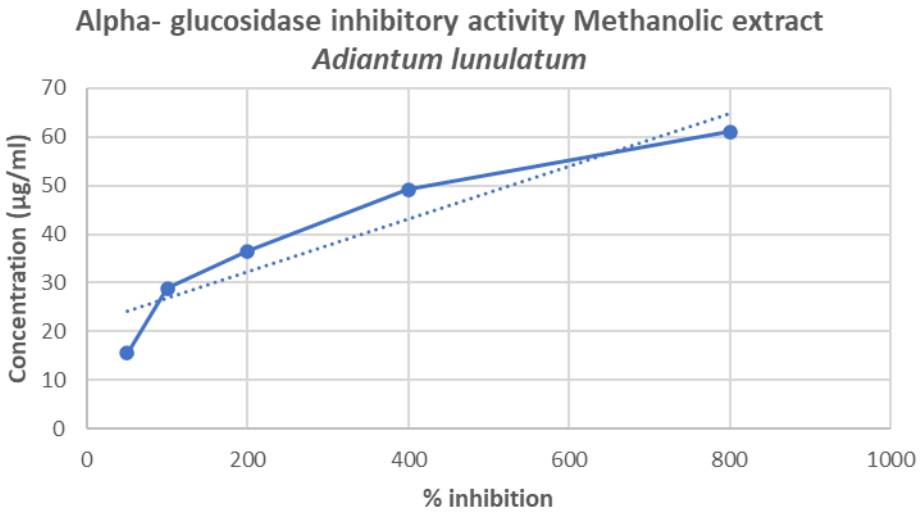

Figure 3. Alpha-glucosidase inhibitory activity of methanol extract of *Adiantum lunulatum*.

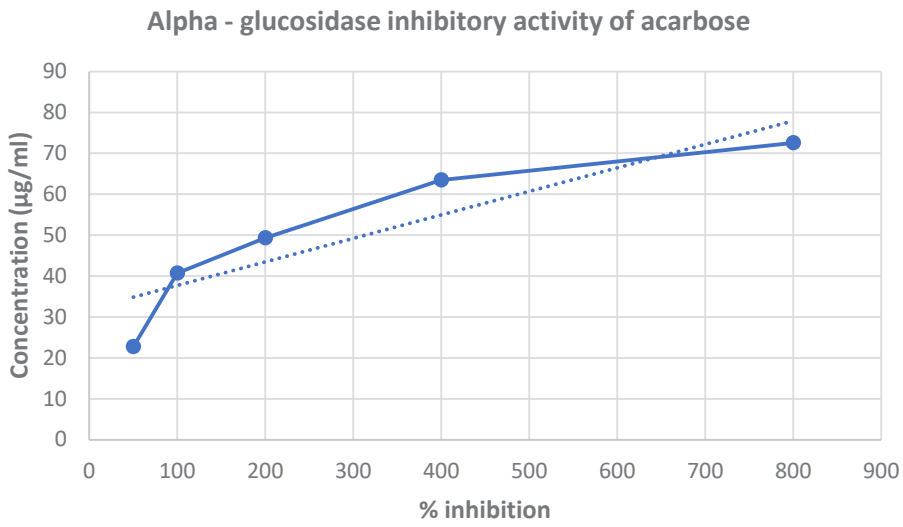

Figure 4. Alpha-glucosidase inhibitory activity of standard acarbose.

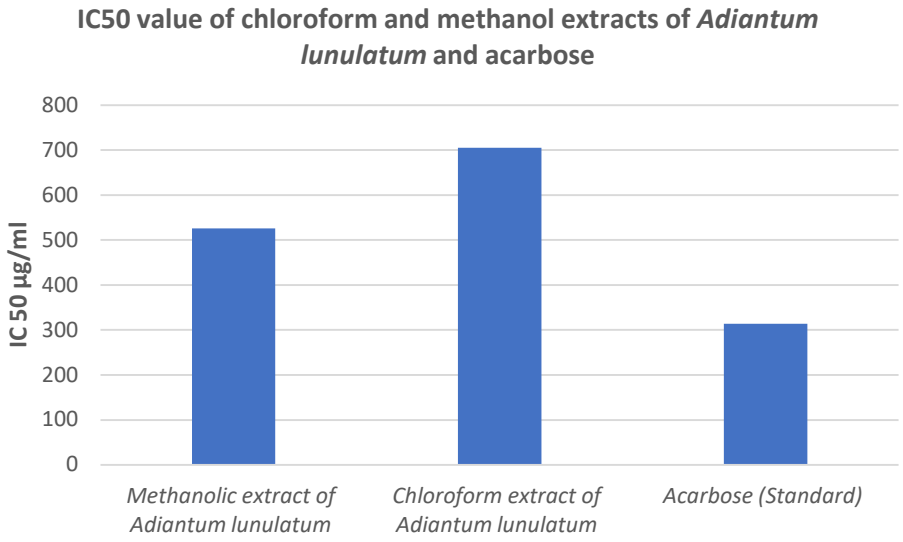

Figure 5. Graph representing the IC_{50} value of chloroform, methanol extracts and standard acarbose.

concluded that methanol has more potent inhibitory activity than chloroform extract and a comparable activity with standard acarbose.

4. Discussion

An increasing number of people around the world are turning to herbal remedies as an adjunct to conventional medicine in the fight against diabetes and its consequences. Various plants from various parts of the world are recognised to possess anti-diabetic properties. When it comes to carbohydrates, lipids and proteins, DM is by far the most prevalent and rapidly expanding metabolic condition in the world[31]. The hallmark of diabetes is poor glucose consumption by tissue, which occurs when insulin secretion is either relative or absolute deficient. Because current modern therapies cannot control all the pathological aspects of the disorder and because these therapies are extremely expensive and not widely available to rural populations in developing countries, there is an immediate need for alternatives to the current modern pharmacotherapy of DM[32].

The main culprits behind postprandial hyperglycemia are α-amylase and α-glucosidase, two enzymes that break down carbohydrates. Alpha-glucosidase converts disaccharides to monosaccharides, resulting in postprandial hyperglycemia, when α-amylase starts the hydrolysis of 1, 4-glycosidic bonds of polysaccharides (such as starch and glycogen) to disaccharides[33]. Therefore, to treat hyperglycemia, α-amylase and alpha-glucosidase inhibitors are helpful because they reduce the postprandial plasma glucose level by delaying carbohydrate digestion[34]. Research on the *A. lunulatum* plant's potential anti-diabetic effects *in vitro* (alpha-glucosidase inhibitory activity) is currently unavailable. This is why we set out to test the alpha-glucosidase inhibitory activity of *A. lunulatum* extracts in chloroform and methanol. Alkaloids, glycosides, flavonoids and tannins were detected in the phytochemical composition of the methanol extract of *A. lunulatum*. These compounds were detected in the chloroform extract: alkaloids, tannins, saponins and flavonoids. One layer of epidermic, sclerenchyma, stele and exarch xylem were identified through microscopic examination of the fronds' rachis, which aids in authenticating. Reducing the rate of glucose absorption and, by extension, the postprandial plasma glucose rise, is possible through blocking alpha-glucosidase activity. A conventional biochemical approach was used to assess the inhibitory action of the test material on alpha-glucosidase. The concentration of test substance required to inhibit 50% of enzyme activity under the conditions was defined as the IC_{50} value. *A. lunulatum* chloroform extract exhibited alpha-glucosidase inhibition activity IC_{50} value of 705.02 ± 2.122 and *A. lunulatum* methanol extract exhibited good alpha-glucosidase inhibition activity with an IC_{50} value, of 526.12 ± 1.155, and standard acarbose 314.11 ± 1.021.

5. Conclusion

To lower the postprandial glucose levels, the current study's results show that the methanol extract of *A. lunulatum* is an effective alpha-glucosidase inhibitor. Nevertheless, additional research is required to identify and define the primary chemicals that block alpha-glucosidase. This could help explore native plant resources for potential novel antidiabetic medicines.

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