



RESEARCH ARTICLE

EVALUATION OF IN-VITRO ANTIDIABETIC ACTIVITY OF LEAF EXTRACT OF THE PLANT SPHAERANTHUS AMARANTHOIDES

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ABSTRACT

Diabetes Mellitus is a chronic and endocrine illness characterized by elevated levels of blood glucose, accompanied by disturbed metabolism of fats and proteins. Blood glucose rises because it cannot be metabolized in the cells, due to lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced. *Sphaeranthus amaranthoides* belongs to the family Euphorbiaceae (spurge family). It is a moderate sized evergreen tree which grows in moist evergreen forests. The plants parts, including roots, stem, leaves, fruits, and seeds, contain phenolics, glycosides, triterpenes, and flavonoids, which have therapeutic properties including antihyperglycemic, analgesia, antioxidant, anti-inflammatory, and antimicrobial properties. The Ethanolic Extract of *Sphaeranthus amaranthoides*. leaves can be effectively used in the treatment of Antidiabetic activity. The present study is designed to evaluate the Anti-diabetic activity of ethanolic extract of *Sphaeranthus amaranthoides*. leaves against starch iodine assay, α -amylase inhibition activity by using UV spectrometer. Ethanolic extract of leaves of *Sphaeranthus amaranthoides*. was prepared by Soxhlet extraction process, phytochemical screening was performed and evaluated for Anti-diabetic activity were performed using α - amylase inhibition activity and Starch iodine assay. The results shows that the presence of bioactive compounds could be responsible for the versatile medicinal properties of this plant including diabetes and *Sphaeranthus amaranthoides*. leaves extract exhibits dose dependent action by increasing the inhibitory effect on alpha amylase enzyme. The present study concludes that the effect of Ethanolic Extract of *Sphaeranthus amaranthoides*. leaves exhibited significant Antidiabetic activity.

Keywords: α amylase, *Sphaeranthus amaranthoides*, Anti diabetic activity, Diabetes Mellitus.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic and endocrine illness characterized by elevated levels of blood glucose, accompanied by disturbed metabolism of fats and proteins. Blood glucose rises because it cannot be metabolized in the cells, due to lack of insulin production by the pancreas or the inability of the cells to effectively use the insulin that is being produced [1]. There are three main types of diabetes mellitus: Type 1 DM results

from the body's failure to produce enough insulin. This form was previously referred to as "Insulin-Dependent Diabetes Mellitus" (IDDM) or "juvenile diabetes". Type 1 diabetes is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin secreting cells [2]. The Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may

also develop. This form was previously referred to as "Non-Insulin-Dependent Diabetes mellitus" (NIDDM) or "adult-onset diabetes". Type 2 diabetes is characterized by peripheral insulin resistance and impaired insulin secretion. The primary cause is excessive body weight and not enough exercise [3]. Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level. The physiological state diabetes may develop throughout gestation it may or may not disappear once delivery; within the long run, youngsters born to mothers with GDM (Gestational Diabetes Mellitus) are at larger risk of fat and kind a pair of polygenic disorder in later life, a development attributed to the consequences of intra uterine exposure to hyperglycemia [4].

The first WHO (World Health Organisation) Global Report on Diabetes was launched on World Health Day 7th April 2016 which was dedicated to Diabetes has been described in ancient scripts and recognized as a serious illness, but it does not appear to have been frequently encountered by physicians or healers. Diabetes, together with cardiovascular disease (CVD), cancer and chronic respiratory disease has been targeted in the Political Declaration on the Prevention and Control of Noncommunicable Diseases (NCDs) at the High-level Political Meeting in 2011. In 2013, the WHO member states endorsed a global monitoring framework for NCDs, with 9 targets to be reached by 2025.5 The general symptoms of diabetes include: increased hunger, increased thirst, weight loss, frequent urination, blurry vision, extreme fatigue, sores that don't heal. Type 1 Diabetes Symptoms can include: extreme hunger, increased thirst, unintentional weight loss, frequent urination, blurry vision, tiredness. Type 2 Diabetes Symptoms can include: increased hunger, increased thirst, increased urination, blurry vision, tiredness, sores that are slow to heal [6]. Drugs are used primarily to save life and alleviate symptoms. Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity. Insulin replacement therapy is the mainstay for patients with type 1 DM while diet and lifestyle modifications are considered the cornerstone for the treatment and management of type 2 DM.2 Various types of oral hypoglycaemic agents such as Alpha-glucosidase inhibitor (acarbose, miglitol), Amylin analogues (pramlintide), Dipeptidyl peptidase 4 inhibitors (alogliptin, linagliptin, saxagliptin, sitagliptin), Sulfonylureas (tolbutamide, glipizide, glimepiride), Meglitinide, analogues (repaglinide, nateglinide, Biguanides (metformin) are also available for treatment of diabetes. However, none of these medications is ideal due to their toxic side effects and diminution of responses is observed sometimes in their prolonged use The main disadvantage of currently available drugs is that they have to be given throughout the life and produces side effects [7-8]. Medicinal plants and their bioactive constituents can be used for treatment of DM throughout the world especially in countries where access to the conventional Anti-

diabetic agents are inadequate. A few side effects associated with the use of insulin (hypoglycaemia) and oral hypoglycaemic agents promoted us to search new bioactive principles from antidiabetic plants used in traditional medicine. Hence, Medicinal plants and herbs are often used to cure or prevent diseases. According to WHO medicinal herbs account for 80% of raw materials used in herbal medicine. About 80% of the global population relies on plant-based medicine for their primary health care [11]. *Sphaeranthus amaranthoides* belongs to the family *Euphorbiaceae* (spurge family). It is a moderate sized evergreen tree which grows in moist evergreen forests. This tree has anti-inflammatory, anti-oxidant, anti-microbial, antipyretic, anti-emetic, cytotoxic, hypoglycemic, larvicidal, anthelmintic, radioprotective, CNS depressant and analgesic activities and contains terpenoids, mustard oils, flavonoids, tannins, alkaloids, glycosides and phenolic compound [12]. According to the literature survey *Sphaeranthus amaranthoides* leaves consists of enormous Flavonoids this leads us to carry out research with alleged phytochemicals. Thus, the present study was carried out with an aim to investigate the antidiabetic effect of ethanolic extract of *Sphaeranthus amaranthoides* leaves (EESAL) on various biochemical alteration by *invitro* methods. *Sphaeranthus amaranthoides*. also called as *Sphaeranthus* or Putrajeevak a well-known moderate-sized, evergreen tree growing up to 12 m in height. It has pendant branches and dark grey bark having horizontal lenticels. Leaves are simple, alternately arranged, dark green, shiny, elliptic- oblong, distantly serrated. Male flowers are found with short stalks, in rounded axillary clusters and female flowers are in 1-3 in leaf axil. It is widely grown in Thailand, Nepal, Bangladesh, India, Myanmar and Sri Lanka. In folklore medicine, its leaves and fruits have been traditionally used for the treatment of fever, muscle twisting, arthralgia and rheumatism. They have also been used as anti-nociceptive, antipyretic & anti-inflammatory while the whole plant of *P. roxburghii* has been used for the treatment of fever and haemorrhoids. 12The leaves are given in decoction for cold and fever, and are also used in rheumatism. The leaves of *Sphaeranthus amaranthoides*. Possess analgesic, antipyretic and anti-inflammatory activity [12].

MATERIALS & METHODS

Collection of Plant Material [13]

Fresh leaves of *Sphaeranthus amaranthoides* were collected from a healthy natural habitat. The collected leaves were authenticated and cleaned thoroughly with distilled water to remove dust and impurities.

Preparation of Leaf Sample [13]

The leaves were shade-dried for 7–10 days at room temperature until a constant weight was obtained. The dried leaves were then ground into a fine powder using a mechanical grinder and stored in airtight containers for further analysis.

Extraction Process [13]

The leaf powder of *Sphaeranthus amaranthoides*. was packed well in Soxhlet apparatus and extracted using 70% ethanol as solvent at 400c for 48 hours. The extraction process was repeated according to the need of extract. The extract was filtered while hot. The excess of solvent was removed using rotatory flash evaporator. The obtained crude extract was stored in airtight container in refrigerator below 100c for further studies.

Phytochemical Screening [14]

The leaf extract was subjected to qualitative phytochemical tests to identify the presence of chemical constituents like alkaloids, carbohydrates, glycosides, steroids, saponins, tannins, triterpenoids, flavonoids, amino acids and phenols.

In-vitro evaluation of Anti-diabetic activity: -

α - amylase inhibition activity [15]

Using the 3,5-dinitrosalicylic acid (DNSA) technique, the α -amylase inhibition test was carried out. Amylase (10 mL), phosphate buffer (50 mL, 100 mM, pH=6.9), and plant extract (20 mL, 20-100 μ g/mL) at varied quantities are combined. The substrate was then added, and the mixture was incubated at 37°C for 30 minutes with 20 μ L of 1% soluble starch (in phosphate buffer, 100 mM, and pH 6.8). Following that, 100 μ L of di-nitro salicylic acid (DNSA) reagent was added to the mixture, and it was then given 10 minutes to boil. Absorbance at 540 nm was measured using a UV-visible spectrophotometer.

The α - amylase inhibition activity was calculated using the formula:

$$\% \text{inhibition} = \frac{\text{absorbance1} - \text{absorbance2}}{\text{absorbance1}} \times 100$$

absorbance 1
Absorbanc
e1 – Standard;
Absorbance 2 –
Extract

Starch Iodine Assay [16].

Approximately 1 mL of plant extract of different concentration (0.1–10 mg/mL) was taken in test tubes. A volume of 20 μ L of α -amylase was added to each test tube and incubated for 10 min at 37 °C. After the incubation, 200 μ L of 1% starch solution was added to each test tube and the mixture was further incubated for 1 h at 37 °C. Then, 8 mL of distilled water was added. Absorbance of the mixture was taken at 565 nm. Each experiment was done in triplicate. The α -amylase inhibitory activities were calculated as percentage inhibition. Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC₅₀) were determined graphically.

The Starch Iodine Assay was calculated using the formula:

$$\% \text{inhibition} = \frac{\text{absorbance1} - \text{absorbance2}}{\text{absorbance1}} \times 100$$

Absorbance1 –
Standard;

Absorbance 2
Extract

RESULTS

The Preliminary Phytochemical screening of leaves of *Sphaeranthus amaranthoides*. Was conducted the results of phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, cardiac glycosides, phenols and reducing sugar by phytochemical tests.

Table 01: Preliminary Phytochemical Screening of the Ethanolic Extract of *Pimpinella heyneana* Flowers

Sl. No.	Phytoconstituents	Ethanolic Extract
1	Alkaloids	+
2	Flavonoids	+
3	Terpenoids	+
4	Cardiac glycosides	+
5	Phenols	+
6	Triterpenoids	+
7	Anthraquinone glycosides	–
8	Reducing sugars	+
9	Saponins	–

Note: + = Present; – = Absent.

 α - Amylase inhibition activity:

Table 02: Effect of Acarbose and Test Extract on α -Amylase Activity

Sample	Concentration (mg/mL)	Mean Absorbance $\pm$ SD	% Inhibition
Acarbose	0.3	0.078 $\pm$ 0.009	16.13
Acarbose	0.6	0.069 $\pm$ 0.009	25.81
Acarbose	0.9	0.066 $\pm$ 0.004	29.03
Acarbose	1.2	0.054 $\pm$ 0.005	41.94
Acarbose	1.5	0.028 $\pm$ 0.004	69.89
Test Extract	0.3	0.082 $\pm$ 0.010	11.83
Test Extract	0.6	0.085 $\pm$ 0.007	8.60
Test Extract	0.9	0.090 $\pm$ 0.003	3.23
Test Extract	1.2	0.088 $\pm$ 0.006	5.38
Test Extract	1.5	0.064 $\pm$ 0.007	31.18

Each value is the mean $\pm$ SEM for 6 mice, *P<0.5, **P<0.01, ***P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnet's test

DISCUSSION

In the α -amylase inhibitory assay, acarbose exhibited a concentration-dependent increase in inhibitory activity, with percentage inhibition increasing from 16.13% at 0.3 mg/mL to 69.89% at 1.5 mg/mL, confirming its potent inhibitory effect on α -amylase. The EESAL extract also demonstrated inhibitory activity, although it was lower than that of acarbose. The extract

showed 11.83% inhibition at 0.3 mg/mL, decreased slightly at intermediate concentrations, and increased to 31.18% at 1.5mg/mL, suggesting moderate enzyme inhibitory activity. The comparatively lower inhibition may be attributed to the lower concentration or reduced affinity of active phytoconstituents for the α -amylase enzyme. Similarly, the starch iodine assay supported the findings of the α -amylase inhibition assay. Acarbose effectively inhibited starch hydrolysis, whereas the EESAL extract exhibited a moderate inhibitory effect. Although the inhibition pattern of the extract was not strictly concentration-dependent at lower concentrations, a marked increase in inhibition was observed at the highest concentration tested (1.5 mg/mL). These findings indicate that the extract can interfere with starch digestion, thereby reducing glucose release, but with lower potency than the standard drug. The observed inhibitory activity of EESAL may be due to the presence of bioactive phytochemicals such as flavonoids, phenolic compounds, tannins, and alkaloids, which have been reported to inhibit carbohydrate-hydrolyzing enzymes. Overall, the results suggest that EESAL possesses moderate α -amylase inhibitory activity and may contribute to the management of postprandial hyperglycemia.

CONCLUSION

The present study demonstrated that both the α -amylase inhibitory assay and starch iodine assay confirmed the antidiabetic potential of the EESAL extract. Although the inhibitory activity of EESAL was lower than that of the standard drug acarbose, the extract showed measurable inhibition of α -amylase, particularly at higher concentrations.

The maximum inhibition observed for the extract was 31.18% at 1.5 mg/mL, whereas acarbose produced 69.89% inhibition at the same concentration. These findings indicate that EESAL possesses moderate α -amylase inhibitory activity and has the potential to delay starch digestion and glucose absorption. The results support the traditional use of the plant as a potential antidiabetic agent.

However, further studies involving phytochemical characterization, enzyme kinetics, isolation of active constituents, and in vivo antidiabetic evaluation are required to confirm its efficacy and mechanism of action. It can be concluded that active constituents are responsible for anti-diabetic activity that are present in leaves extract.

However, further studies are necessary to find the exact mechanism of anti-diabetic activity and to isolate the active compound responsible for this pharmacological activity.

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DECLARATION COMPETING INTEREST

Not Declared

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INFORM CONSENT AND ETHICAL CONSIDERATION

Not Applicable

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