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***In Vitro* Alpha Amylase and Alpha Glucosidase Inhibitory Activity of Important Lettuces from South Indian Region**

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Abstract: Alpha amylase and alpha glucosidase inhibition were investigated in this *in vitro* investigation utilising ethanolic extract of the leaves of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum*, and *Spinacia oleracea*. The inhibition of alpha amylase was seen for *Moringa oleifera* (82.9 %), *Sesbania grandiflora* (80.48 %), *Spinacia oleracea* (80.48 %) and *Solanum nigrum* (76.42 %), from their respective ethanolic extracts, when compared to the reference medication acarbose (89.43 %). *Sesbania grandiflora* (69.49%), *Moringa oleifera* (83.15%), *Solanum nigrum* (66.10%), and *Spinacia oleracea* (66.94%) all inhibited alpha glucosidase at a concentration of 100 µg/ml, whereas the standard acarbose medication inhibited alpha glucosidase at 89.83%. The ethanolic extract of leaves from herbs is thus proposed as a possible source for natural antidiabetic and antioxidant chemicals with use in the treatment of diabetes mellitus.

Keywords: Alpha amylase, Alpha glucosidase, Ethanolic extract, Standard acarbose drug, Antidiabetic, Antioxidant

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Introduction

Chronic metabolic condition characterised by abnormal lipid, protein, and carbohydrate metabolism; medically known as diabetes mellitus (Vijayamuthuramalingam *et al.*, 2017). This is caused by high blood glucose (blood sugar) levels. On the other hand, hypoglycemia occurs when blood glucose levels drop too low.

Diabetes comes in three primary forms (Khan *et al.*, 2006; Zaid *et al.*, 2012). Type I diabetes, often known as juvenile diabetes, is caused by an insufficient amount of insulin being produced. In those with Type II diabetes, either insulin production is inadequate or insulin resistance is present. Gestational diabetes, or type III diabetes,

is caused by the body's resistance to insulin hormones (Kirwan *et al.*, 2016). Diabetic treatment focuses on increasing insulin production, decreasing insulin requirements, and improving insulin's function. Drugs that limit alpha-glucosidase activity, such as acarbose, reduce the rate at which starch is converted into glucose, therefore preventing or delaying a rise in blood sugar levels (Kirwan *et al.*, 2016; Moein *et al.*, 2017). Transformation of starch into simpler sugars is a result of the inhibitory action of α -amylase enzymes. These inhibitors slow the rate at which glucose is absorbed into the bloodstream, helping to keep serum blood glucose levels constant (Apostolids *et al.*, 2007). Inhibitors of α -amylase and α -glucosidase enhance postprandial blood glucose excursion and decrease diabetes risk (Grover *et al.*, 2002).

Glucose binding to the blood without reducing blood sugar is the result of inhibitors like DPPH - 4, which include alogliptin, linagliptin, and saxagliptin. Herbal medicine as a discipline has expanded rapidly in recent decades. Because of its natural nature, it has no adverse effects and costs less than synthetic hypoglycemic agents (Sheikh *et al.*, 2015).

The hummingbird tree, also known as an ageate, is a species of *Sesbania grandiflora* which belongs to Fabaceae family. Analysis of the extract was performed to dilute the many elements present, such as antioxidant activity, so that the pharmacological activity of the plant could be determined. Wound healing activity was found in the leaf extract, and antibacterial activity was found in the leaves, stems, gnanules, and pods of this medicinal plant (Matta-Toreres *et al.*, 2021). The chenopodiaceae family is home to *Spinacia oleracea*. This healing plant is composed of a number of different chemical compounds. Some of the flavonoids said to be present include quercetin, myricetin, and kampeferal. This medicinal plant's pharmacological properties included antitumor activity (Ramesh *et al.*, 2010). The "Black night shade," or *Solanum nigrum*

possess numerous therapeutic effects, including antimicrobial, antioxidative, cytotoxic and antiulcerogenic. Analyzing this extract for its ability to identify different elements might provide information about the plants' pharmacological screening actions. The leaves of this plant have a long history of medicinal usage, including for conditions such as rheumatism, gout, skin disorders, tuberculosis, and neurological disorders. The anti-cancer properties (Lee *et al.*, 2014) of *Solanum nigrum* are its most valuable attribute. *Moringa oleifera* is a versatile plant that may be used for food, medicine, and even fuel. It is included in the genus *Moringa* and the family Moringaceae. Leaf extracts of *Moringa oleifera* include carotenoids and critical amino acids. *Moringa oleifera* is loaded with beneficial elements including vitamins and minerals as well as antioxidants and anti-infectives. This plant has been shown to have anti-diabetic and antioxidant effects (Khare *et al.*, 2007).

Sesbania grandiflora's antidiabetic effect has been reported at the concentration of 100 $\mu\text{g/ml}$, α amylase was inhibited by 81%, which is lower than the 93% inhibition shown with the acarbose standard (Gupta *et al.*, 2018). Based on comparisons with acarbose, the highest inhibition for α amylase (80.50%) and α glucosidase (75.65%) was seen with 200 $\mu\text{g/ml}$ of an aqueous extract of *Moringa oleifera* leaves (Sultana and Anwar, 2008). Maximum inhibitory effect on α amylase were estimated to be 743.7 $\mu\text{g/ml}$ as compared with the standard acarbose at 449.4 $\mu\text{g/ml}$ and α glucosidase were estimated to be 254.70 $\mu\text{g/ml}$ as compared with the standard acarbose at 441.0 $\mu\text{g/ml}$ (Maeda *et al.*, 2008).

In contrast to the standard drug glinil, which exhibits a rate of glucose inhibition ranging from 51% to 26%, the anti-diabetic activity of *Solanum nigrum* was increased by using the ethanoic extract, with the maximum inhibition for α amylase increasing from 37% to 88% with increasing concentration (Sharifi-Rad *et al.*,

2017). Studies on the anti-oxidant and anti-diabetic properties of *Spinacia oleracea* leaves using methanolic extract have shown that the maximal inhibition occurs at a concentration of 211081.58 µg/ml, whereas the corresponding value for ascorbic acid is 14.15 µg/ml (Hossain *et al.*, 2020).

In vitro α-amylase and α-glucosidase inhibitory properties of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum*, and *Spinacia oleracea* ethanolic extracts were studied in the present study.

Materials and Methods

Preparation of plant extract:

The plants of *Sesbania grandiflora*, *Spinachia oleracea*, *Moringa oleifera* and *Solanum nigrum* were collected in the month of December from Trichy, Tamil Nadu, India. *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum*, and *Spinacia oleracea* were cleaned, chopped, and dried at 35-40 °C. It was then ground to 40 mesh powder. Hot-Percolation of 100 g of dry powder of plant materials in 1 L of ethanol yields the extract. The extract is filtered using Whatmann filter paper 42 after 10 h (125 mm). Extracts were dried by using Reduced-pressure rotary evaporators. Samples were dried at 40 °C and stored in Labeled sterile vials.

Anti-diabetic activity:

Alpha-amylase inhibitory assay:

250 µl of ethanolic extract (20-100 µg/ml) was poured in a tube with 250 µl of 0.02M sodium phosphate buffer and α-amylase solution (0.5 mg/l) (pH 6.9). The solution was pre-incubated for 10 min at 20 °C with 1% starch in 0.02 M sodium phosphate buffer (pH 6.9) and terminated with 500 µl of dinitrosalicylic acid (DNS) reagent. The tubes were incubated with boiling water for 5 min and cooled at room temperature before dilution with 5 ml distilled water and read at spectrophotometer absorbance at 540 nm. The α-amylase inhibitory activity was calculated as percentage inhibition:

$$\% \text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extracts}}}{\text{Abs}_{\text{control}}} \right] \times 100.$$

The resulting of extracts in 50% inhibition of enzyme activity (IC₅₀) determined graphically.

Alpha-glucosidase inhibitory assay:

The analysis of α-glucosidase activity was performed by method of Kim *et al.* (2005). p-NPG was pre-incubated with 50 µl of varied concentrations in 20 mM phosphate buffer (pH 6.9, 100 µl of α-glucosidase (1.0 U/ml) and 20–100 µg/ml ethanolic extract). 2 ml of 0.1M Na₂CO₃ terminated the 20 min incubation at 37 °C. Yellow-colored p-nitro phenol from p-NPG at 450 nm measured glucosidase activity.

The α-glucosidase inhibitory activity was calculated as percentage inhibition:

$$\% \text{Inhibition} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extracts}}}{\text{Abs}_{\text{control}}} \right] \times 100.$$

The resulting of extracts in 50% inhibition of enzyme activity (IC₅₀) determined graphically.

Results and Discussion

Modern studies have shown not only the efficacy of plants as diabetes therapies, but also the mechanism by which they work. Diabetes has historically been treated using plants. For example, oxidation and inflammation, both of which have been recently related to the development and worsening of the overall diabetic state (Alqahtani *et al.*, 2019) are two conditions that may be treated and prevented by the direct action of plant metabolites. They may also have an indirect effect by causing cells to secrete more insulin and therefore reducing glucose synthesis and absorption.

One of the main goals of diabetes treatment is to reduce or eliminate glucose absorption. By inhibiting the digestive enzymes responsible for breaking down polysaccharides into tiny, absorbable parts, we may prevent postmeal high blood sugar. Examples of inhibitors belonging to this class include glucosidase and amylase inhibitors.

Table 1: In vitro antidiabetic activity of the leaves of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum* and *Spinacia oleracea* comparison with standard acarbose drug using alpha amylase method

S. No.	Concentration	Alpha amylase %				
		<i>Sesbania grandiflora</i>	<i>Moringa oleifera</i>	<i>Solanum nigrum</i>	<i>Spinacia oleracea</i>	Acarbose
1.	20 (µg/ml)	51.21	64.22	52.03	58.53	82.11
2.	40 (µg/ml)	61.78	73.98	63.41	60.97	83.74
3.	60 (µg/ml)	68.29	77.23	69.91	73.98	85.34
4.	80 (µg/ml)	77.23	81.30	73.17	75.04	89.43
5.	100 (µg/ml)	80.48	82.98	76.42	80.48	89.43

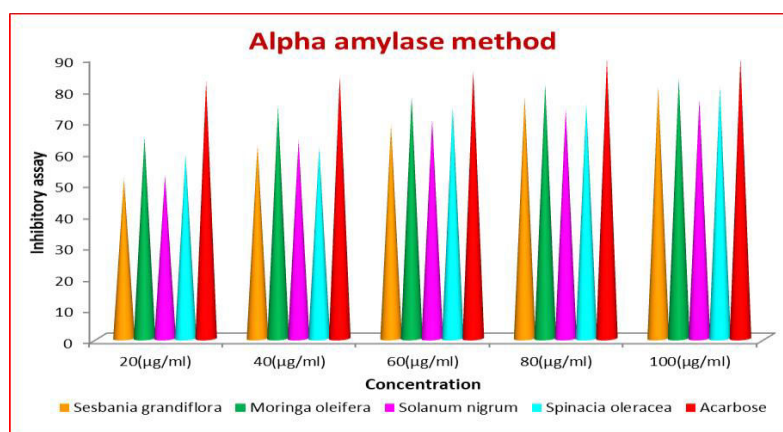


Fig. 1: Alpha amylase inhibitory activity of Acarbose VS leaves of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum* and *Spinacia oleracea*.

In vitro alpha amylase inhibitory assay:

Since it plays a substantial role in the breakdown of polysaccharides, alpha-amylase, which is mostly present in saliva and pancreatic juice, is considered one of the most important enzymes in the digesting process. Targeting and inhibiting this enzyme might be a promising strategy for reducing postmeal blood sugar levels that rise too high (Rujanapun *et al.*, 2022).

Alpha amylase, an essential enzyme secreted by the pancreas, converts starch into glucose, the body's preferred sugar. However, this enzyme may be harmful for those with diabetes since it causes an increase in blood sugar levels. The clearance zone was much smaller in the well test compared to the control group. Based on these results, it seems that the extract has a substantial inhibitory effect on alpha amylase. The DNS method (Fig. 1) shows that the plant extract inhibits the α -amylase enzyme at increasing

concentrations. As a precaution, this might stop starch from being turned into glucose and so keep blood sugar levels stable (Choudhury *et al.*, 2017).

In vitro alpha amylase inhibitory properties of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum*, and *Spinacia oleracea* ethanolic extracts were studied. *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum*, and *Spinacia oleracea* (20-100 µg/ml) have dose-dependent α -amylase inhibition. *Sesbania grandiflora* (80.48%), *Moringa oleifera* (82.98%), *Solanum nigrum* (76.42%), and *Spinacia oleracea* (80.48%) inhibited acarbose (89.43%) at 100 µg/ml (Table 1). *In vitro* α -amylase inhibition assessed the isolated compounds for antibacterial potential.

In vitro α -glucosidase inhibitory assay:

The mucosal brush border of the small intestine houses the α -glucosidase enzyme, another crucial digestive enzyme. It helps to digest complex

Table 2: *In vitro* antidiabetic activity of the leaves of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum* and *Spinacia oleracea* comparison with standard acarbose drug using alpha glucosidase method

S. No.	Concentrations	Alpha glucosidase (%)				
		<i>Sesbania grandiflora</i>	<i>Moringa oleifera</i>	<i>Solanum nigrum</i>	<i>Spinacia oleracea</i>	Acarbose
1.	20(μ g/ml)	58.47	67.79	55.08	57.62	83.05
2.	40(μ g/ml)	63.55	68.64	58.47	60.16	85.59
3.	60(μ g/ml)	65.25	75.42	60.16	62.71	86.44
4.	80(μ g/ml)	67.79	78.81	62.71	65.25	88.13
5.	100(μ g/ml)	69.49	83.05	66.10	66.94	89.83

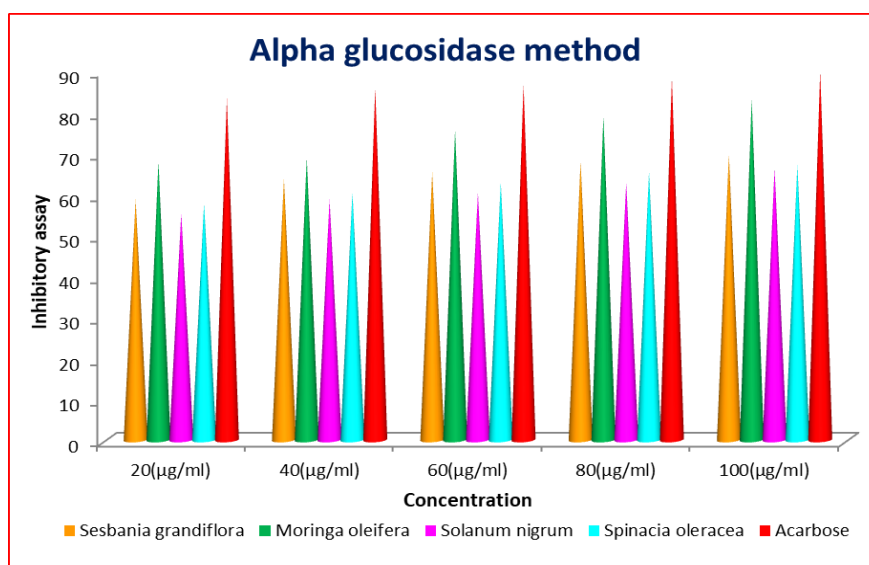


Fig. 2: Alpha glucosidase inhibitory activity of Acarbose VS leaves of *Sesbania grandiflora*, *Moringa oleifera*, *Solanum nigrum* and *Spinacia oleracea*.

carbohydrates by converting them into simpler sugars. Delaying glucose absorption and reducing high postprandial blood glucose levels by its suppression is an effective strategy, and may help reduce the progression of diabetes (Mechchate *et al.*, 2021). The ethanolic extract of the leaves of *Sesbania grandiflora* (69.49%), *Moringa oleifera* (83.15%), *Solanum nigrum* (66.10%), and *Spinacia oleracea* (66.94%) showed considerable inhibitory effect of the α -glucosidase enzyme when compared to the standard medication acarbose (89.83%) (Table 2). Increasing extract

concentrations at 100 μ g/ml increased the percentage of inhibition (Fig. 2). The α -glucosidase inhibitor acarbose has also been widely prescribed. It shows that leaves are a far more powerful inhibitor of alpha-amylase and alpha-glucosidase than acarbose.

Conclusion

Inhibition of alpha amylase and alpha glucosidase using ethanolic extracts of leaves from *Moringa oleifera*, *Sesbania grandiflora*, *Solanum nigrum* and *Spinacia oleracea* was shown to be most

effective compared to the gold standard medication acarbose. When compared to the gold standard medicine acarbose, *Moringa oleifera* demonstrated the highest inhibitory activity of (83.05%), followed by the leaves of *Sesbania grandiflora*, *Solanum nigrum*, and *Spinacia oleracea*. The results of our research indicated that the inhibitory action of the ethanolic extract of these medicinal herbs against alpha glucosidase and alpha amylase may be used to treat type 2 diabetes mellitus.

References

- Alqahtani AS, Hidayathulla S, Rehman MT, ElGamal AA, Al-Massarani S, Razmovski-Naumovski V, Alqahtani MS, El Dib RA and Al Ajmi MF. (2019) Alpha-amylase and alpha-glucosidase enzyme inhibition and antioxidant potential of 3-oxolupenal and katononic acid isolated from *Nuxia oppositifolia*. *Biomolecules* 10(1): 61.
- Apostolidis E and Shetty E. (2007) Evaluation of pepper (*Capsicum annuum*) for management of diabetes and hypertension. *J Food Biochem*. 31(3): 370-385.
- Choudhury H, Pandey M, Hua CK, Mun CS, Jing JK, Kong L, Ern LY, Ashraf NA, Kit SW, Yee TS, Pichika MR, Gorain B and Kesharwani P. (2017) An update on natural compounds in the remedy of diabetes mellitus: A systematic review. *J Tradit Complement Med* 8(3): 361-376.
- Grover JK, Yadav S and Vats V. (2002) Medicinal plants of India with anti-diabetic potential. *J Ethnopharmacol* 81(1): 81-100.
- Gupta RA, Motiwala MN, Mahajan UN and Sabre SG. (2018) Protective effect of *Sesbania grandiflora* on acetic acid induced ulcerative colitis in mice by inhibition of TNF- α and IL-6. *J Ethnopharmacol*. 219: 222-232.
- Hossain U, Das AK, Ghosh S and Sil PC. (2020) An overview on the role of bioactive α -glucosidase inhibitors in ameliorating diabetic complications. *Food Chem Toxicol*. 145: 111738
- Hsiu J, Fischer EH and Stein EA. (1964) Alpha-amylases as calcium-metalloenzymes. ii. calcium and the catalytic activity. *Biochemistry* 3: 61-66.
- Kahn SE, Hull RL and Utzschneider KM. (2006) Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 444(7121):840-846.
- Khare CP. (2007) *Indian Medicinal Plants: 1st edn.*, Springer Verlag, Berlin/Heidelberg.
- Kirwan JP, Aminian A, Kashyap SR, Burguera B, Brethauer SA and Schauer PR. (2016) Bariatric surgery in obese patients with type 1 diabetes. *Diabetes Care* 39(6): 941-948.
- Lee JH, Cho S, Paik HD, Choi CW, Nam KT, Hwang SG and Kim SK. (2014) Investigation on antibacterial and antioxidant activities, phenolic and flavonoid contents of some thai edible plants as an alternative for antibiotics. *Asian-Australas J Anim Sci*. 27(10): 1461-1468.
- Maeda N, Kokai Y, Ohtani S, Sahara H, Kumamoto-Yonezawa Y, Kuriyama I, Hada T, Sato N, Yoshida H and Mizushima Y. (2008) Anti-tumor effect of orally administered spinach glycolipid fraction on implanted cancer cells, colon-26, in mice. *Lipids* 43(8): 741-748.
- Mata-Torres G, Andrade-Cetto A and Espinoza-Hernández F. (2012) Approaches to decrease hyperglycemia by targeting impaired hepatic glucose homeostasis using medicinal plants. *Front Pharmacol* 12: 809994.
- Mechchate H, Es-Safi I, Louba A, Alqahtani AS, Nasr FA, Noman OM, Farooq M, Alharbi MS, Alqahtani A, Bari A, Bakkari H and Bousta D. (2021) *In Vitro* alpha-amylase and alpha-glucosidase inhibitory activity and in vivo antidiabetic activity of *Withania frutescens* L. foliar extract. *Molecules* 26(2): 293.
- Moein S, Pimoradloo E, Moein M and Vessal M. (2017) Evaluation of antioxidant potentials and α -amylase inhibition of different fractions of Labiatae plants extracts: as a model of antidiabetic compounds properties. *Biomed Res Int*. 2017: 7319504.
- Ramesh T, Sureka C, Bhuvana S and Hazeena Begum V. (2010) *Sesbania grandiflora* diminishes oxidative stress and ameliorates antioxidant capacity in liver and kidney of rats exposed to cigarette smoke. *J Physiol Pharmacol*. 61(4): 467-476.
- Rujanapun N, Jaidee W, Duangyod T, Phuneerub P, Paojumroom N, Maneerat T, Pringpuangkeo C, Ramli S and Charoensup R. (2022) Special Thai Oolong tea: Chemical profile and *in vitro* antidiabetic activities. *Front Pharmacol*. 13: 797032.
- Sharifi-Rad J, Sureda A, Tenore GC, Daglia M, Sharifi-Rad M, Valussi M, Tundis R, Sharifi-Rad M, Loizzo MR, Ademiluyi AO, Sharifi-Rad R, Ayatollahi SA and Iriti M. (2017) Biological activities of essential oils: From plant chemoecology to traditional healing systems. *Molecules* 22(1):70.
- Sheikh Y, Maibam BC, Biswas D, Laisharm S, Deb L, Talukdar NC and Borah JC. (2015) Anti-diabetic potential of selected ethno-medicinal plants of north east India. *J Ethnopharmacol*. 171: 37-41.

Sultana B and Anwar F. (2008) Flavonols (kamafeferol quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants: Food Chem. 108: 879-884.

Vijayamuthuramalingam UDK, Rajaram R, Kuppusamy KM, Jonnalagadda B and Arokiasamy S. (2017) Anti-hyperglycemic and antioxidant potential of *Croton bonplandianus*. Bail fractions in correlation with polyphenol content. Iran J Basic Med Sci. 20(12): 1390-1397.

Zaid MA, Sharma KK and Rizvi SI. (2002) Effect of (-)epicatechin in modulating calicum-ATPase activity in normal and diabetic human erythrocytes. Indian J Clin Biochem. 17(2): 27-32.