Introduction

Diabetes mellitus is a chronic metabolic disorder, characterized by hyperglycemia and carbohydrate, protein, and fat metabolic disturbances. It causes failing of insulin production or insulin action or both. According to an estimation of the International Diabetes Federation, approximately 366 million people are suffering from diabetes and this may double by 2030, in India to be 40.9 million, which is expected to grow to 60.9 million by 2025. Between two types of diabetes, type 2 is more prevalent than type 1, with more than 90% of the total diabetic patients suffering from it. Type 2 diabetes (T2D) is a disease caused by an imbalance between blood sugar absorption and insulin secretion. Postprandial hyperglycemia plays an important role in the development of T2D. Regulating plasma glucose level is vital for delaying or preventing T2D. The ability of a drug or diet to delay the production or absorption of glucose by inhibiting carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase is one of the therapeutic goals in the treatment of T2D.

ABSTRACT

Objective: The objective of the present study was to provide an in-vitro evidence for the potential inhibitory activity of extracts and fractions of Adiantum caudatum Linn. and Celosia argentea Linn. on α-amylase and α-glucosidase enzymes.

Materials and Methods: The plant extracts were prepared, first with cold maceration (70% v/v ethanol) and then by Soxhlation techniques (95% v/v ethanol). Subsequently, the combined extracts were subjected for fractionation. Different concentrations (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) of extract and fractions were subjected to α-amylase and α-glucosidase inhibitory assay. The absorbance was measured at 540 and 405 nm using multichannel reader and the percentage of α-amylase and α-glucosidase inhibitory activity and IC50 values of extract and fractions were calculated.

Results: Fraction 2 of A. caudatum and fraction 4 of C. argentea has shown highest α-amylase and α-glucosidase inhibitory potential with IC50 values of 0.241, 0.211 and 0.294, 0.249 mg/ml, respectively, which was comparable with acarbose (0.125 and 0.93 mg/ml). Whereas, extracts and remaining fractions of both the plants have shown lesser activity.

Conclusion: The results of the present study indicate that, fraction 2 of A. caudatum, rich in triterpenoids and phenolics and fraction 4 of C. argentea, rich in flavonoids, are effective α-amylase and α-glucosidase inhibitors, which may be helpful to reduce the postprandial glucose levels. Hence, further studies may throw light on the antidiabetic potential of A. caudatum and C. argentea, especially in the management of type 2 diabetes.

KEY WORDS: α-amylase, α-glucosidase, Mayurasikha, type 2 diabetes
approaches for decreasing postprandial hyperglycemia. At present, the use of insulin secretagogues and sensitizers constitute the predominant line of therapy; however, the use of carbohydrate digesting enzyme inhibitors play a vital role in controlling hyperglycemia by reducing the intestinal absorption of glucose. Acarbose is one of the leading inhibitor of carbohydrate metabolic enzymes in the gastrointestinal tract, but it is associated with side effects such as diarrhea and other intestinal disturbances such as bloating, flatulence, cramping, and abdominal pain. World Health Organization (WHO) estimates that about three-quaters of the population mainly in the countries of Africa, Asia, and Latin America, confines on plant based preparations in their traditional medicinal system for primary healthcare (WHO, 2003). This dependence increased the knowledge gathering and exploration of novel and effective plant-derived compounds for commercialization. Predominantly herbal drugs have been widely used globally for diabetic treatment over thousands of years due to their traditional acceptability and lesser side effects. Therefore, screening of α-amylase and α-glucosidase inhibitors in medicinal plants has received much attention.

Ayurveda and other Sanskrit literature describe Mayurasikha as a Sandigdha dravya (controversial drugs). Which having more than one botanical source. Dravyaguna Vijnana by P.V. Sharma named Adiantum caudatum as Mayurasikha and Shaligramanighantubhushanan describes Celosia argentea (syn-celosia cristata) as Mayurasikha. Hence, these two plants were selected for comparative evaluation of α-amylase and α-glucosidase inhibitory activity.

A. caudatum Linn. (Pteridaceae) is an fern, which is available especially lower slopes of the hills in Punjab, Rajasthan, West Bengal, Tamil Nadu, and Maharashtra. Ayurveda describes that it would be useful treat Prameha (diabetes), Atisara, Pravahika, cough, skin diseases, and fever. C. argentea Linn. (Amaranthaceae) is annual herb (0.5–1.5 m), a common weed, occurring throughout India. In Indian folk medicine, it was used for diabetes and the seeds were used in the treatment of jaundice, gonorrhea, wounds, and fever.

There are scanty reports available regarding the phytoconstituents responsible for inhibiting the carbohydrate digestive enzymes, which can able to manage diabetes mellitus. Hence, the main objective of present study was to investigate in-vitro, α-amylase, α-glucosidase inhibitory activity of the hydro-alcoholic extract, and fractions of A. caudatum and C. argentea.

Materials and Methods

Chemicals
α-glucosidase (Saccharomyces cerevisiae), α-amylase (procaine pancreas) and 3, 5, di-nitro salicylic acid (DNS) were purchased from Sigma-Aldrich, Bangalore. P-nitro-phenyl-α-D-glucopyranoside (p-NPG), sodium carbonate (Na₂CO₃), sodium dihydrogen phosphate, di-sodium hydrogen phosphate were purchased from Hi-Media, Mumbai.

Plant Material
A. caudatum and C. argentea were collected during the month of August 2013 from adjoining areas of Visvesvaraya Technological University, Belagavi, Karnataka. Authentication of the plants was done by Dr. Harsha Hegde, Scientist C (RMRC, Belgaum), and a voucher specimens (RMRC-985, 987) was deposited at RMRC (ICMR), Belagavi. The plant material was washed under running tap water and dried under shade, coarsely powdered (#2000/335), and stored in the neatly labeled airtight container.

Extraction and Fractionation
Dried powdered (500 g) material was first subjected to cold maceration to extract thermostable constituents if any with 70% v/v ethanol for 24 h. Extract was filtered, and the marc was further subjected for soxhlation (95% v/v ethanol). Filtrates of both maceration and soxhlation were combined and concentrated using a rotary evaporator (IKA RV 10) at 40°C under reduced pressure, which yields total extract of 40 g and 46 g.

Fractionation of A. caudatum extract was carried out as per Cos et al., with minor modifications [Figure 1]. Alcoholic extract was dispersed in 5% w/v citric acid and washed with dichloromethane. Dichloromethane layer was separated and it was concentrated to 1/3rd volume using rotary evaporator at 40°C under reduced pressure. Concentrated dichloromethane layer was partitioned with 90% v/v methanol and petroleum ether (1:1) to get fraction 1, (F1, 9.65 g) and fraction 2, (F2, 7.15 g). Aqueous layer was concentrated to half and pH adjusted to 9.0 with 10% ammonium hydroxide. Aqueous layer was washed with dichloromethane, which gives fraction 3 and fraction 4 (F3, 0.584, and F4, 16.45 g). Same procedure was used for fractionation of C. argentea extract and percentage yield of the fractions were F1 10.15 g, F2 9.76 g, F3 0.593 g, and F4 18.65 g, respectively.

In-vitro Assay
α-amylase inhibitory activity
α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification. In a 96-well plate, reaction mixture containing 50 μl phosphate buffer (100 mM, pH = 6.8), 10 μl α-amylase (2 U/ml), and 20 μl of varying concentrations of extract and fractions (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was preincubated at 37°C for 20 min. Then, the 20 μl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 μl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture
was measured at 540 nm using Multiplate Reader (Multiska thermo scientific, version 1.00.40). Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. Without test (extract and fractions) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

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

Where,

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

**α-glucosidase inhibitory activity**

α-glucosidase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification.\(^{12}\) In a 96-well plate, reaction mixture containing 50 μl phosphate buffer (100 mM, pH = 6.8), 10 μl α-glucosidase (1 U/ml), and 20 μl of varying concentrations of extract and fractions (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) was preincubated at 37°C for 15 min. Then, 20 μl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 μl Na₂CO₃ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

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

Where,

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

**Statistical Analysis**

All the measurements were done in triplicate and results are expressed in terms of mean ± standard deviation and IC₅₀ values were calculated using GraphPad Prism 5 version 5.01 (Graphpad software, Inc., La Jolla, CA, USA.) statistical software.

**Results**

In the present study, hydro-alcoholic extract and four fractions of *A. caudatum* and *C. argentea* were evaluated for their inhibitory effect on α-amylase and α-glucosidase enzymes by *in-vitro* method. The hydro-alcoholic extract and its fractions 1, 2, 3, and 4 of *A. caudatum* (at a concentration of 0.5 mg/ml) exhibited 32.42, 46.25, 61.45, 20.12, and 26.04 α-amylase inhibitory activity [Figure 2] and 36.42, 47.25, 63.45, 29.22, and 32.34 α-glucosidase inhibitory activity [Figure 3], respectively. Whereas, *C. argentea* hydro-alcoholic extract and Fractions 1, 2, 3, and 4 exhibited 22.42, 21.04, 29.04, 30.12, and 59.45 α-amylase inhibitory activity [Figure 4], respectively. Whereas, *C. argentea* hydro-alcoholic extract and Fractions 1, 2, 3, and 4 exhibited 22.42, 21.04, 29.04, 30.12, and 59.45 α-amylase inhibitory activity [Figure 4], respectively. *C. argentea* showed best enzyme inhibitory activity with an IC₅₀ value 0.249 mg/ml (α-amylase and α-glucosidase) [Table 2] which were comparable with that of acarbose.

**Figure 2: α-amylase inhibition of *Adiantum caudatum* extract and fractions**

![Figure 2](image1.png)

**Figure 3: α-glucosidase inhibition of *Adiantum caudatum* extract and fractions**

![Figure 3](image2.png)

**Figure 4: α-amylase inhibition of *Celosia argentea* extract and fractions**

![Figure 4](image3.png)
Discussion

The use of herbal drugs as complementary approaches in existing medications for the treatment of diabetes and its complications is growing worldwide and many plants in different countries are known to have antidiabetic effects.[23] The ancient Indian literature reports more than 800 plants with antidiabetic properties while ethnopharmacological surveys indicate that more than 1200 plants can be used for hypoglycemic activity.[14] Mainly two carbohydrate hydrolyzing enzymes (α-amylase and α-glucosidase) are responsible for postprandial hyperglycemia. α-amylase begins the process of carbohydrate digestion by hydrolysis of 1, 4-glycosidic linkages of polysaccharides (starch, glycogen) to disaccharides and α-glucosidase catalyzes the disaccharides to monosaccharides, which leads to postprandial hyperglycemia.[15,16] Hence, inhibitors of α-amylase and α-glucosidase are useful in the control of hyperglycemia as they delay carbohydrate digestion, which consequently reduce the postprandial plasma glucose level. Previously, the antidiabetic activity of A. caudatum and C. argentea has been reported in the literature. The ethanolic extract of A. caudatum whole plant shown antidiabetic activity with 200 mg/kg b.w in streptozotocin-induced diabetic rats. Ethanolic extract of C. argentea roots and seeds with 250 and 500 mg/kg b.w reduced hyperglycemia in streptozotocin and alloxan-induced diabetic rats.[17-19] There was no information available in the literature about the in-vitro (α-amylase and α-glucosidase inhibitory activity) antidiabetic studies of these two plants. Hence, the present study aimed to evaluate α-amylase and α-glucosidase inhibitory activity of hydro-alcoholic extract and its fractions of A. caudatum and C. argentea. Alkaloids, phenolics, triterpenoids, flavonoids, and steroids were identified in the preliminary phytochemical investigation of A. caudatum. After fractionation of the hydro-alcoholic extract, steroids and lipids were found in fraction 1, phenolics and terpenoids were found in fraction 2. Fraction 3 was shown positive results for alkaloids and fraction 4 shown positive results for flavonoids. Based on the results obtained, fraction 2 was showed highest inhibitory potential than extract and other fractions. Whereas, phytochemical investigation of C. argentea showed positive results for alkaloids, phenolics, triterpenoids, flavonoids, steroids, saponins, and tannins. After fractionation of the hydro-alcoholic extract, steroids, and lipids were found in fraction 1, phenolics, and terpenoids were found in fraction 2. Fraction 3 was shown positive results for alkaloids and fraction 4 shown appreciable levels of flavonoids. Based on the results obtained, fraction 4 of C. argentea shown highest inhibitory potential than extract and other fractions. Many bioactive compounds from different plants have been reported to have hypoglycemic effect, in that mostly phenolics and triterpenoids such as oleanane, ursane, lupane, and flavonoids have a positive correlation as antidiabetic agents.[20-22] The presence of triterpenoids and phenolics in fraction 2 might have attributed to the highest enzyme inhibition activity compared to other fractions in A. caudatum.[23] Hence, the triterpenoids of this plant may be responsible for enzyme inhibitory activity. Apart from that polyphenolic compounds were found in fraction 2, may interact or inhibit specific positions in enzymes thereby reducing the potency of α-amylase and α-glucosidase.[24] The presence of flavonoid compounds in fraction 4 of C. argentea may act against diabetes mellitus either through their capacity to avoid glucose absorption or to improve glucose tolerance by competitive inhibition of sodium-dependent glucose transporter-1.[25] Another possible mechanism followed by flavonoid compounds (luteolin, kaempferol, chrysin, and galangin) to control blood glucose levels is the inhibition of α-amylase and α-glucosidase activity in the intestine.[26] Due to above reasons, fraction 2 of A. caudatum and fraction 4 of C. argentea showed comparable results with that of acarbose. With the help of results in correlation with previous reports it can be hypothesized that the significant enzyme inhibitory activity of fraction 2 and fraction 4 may interfere or delay the absorption of dietary carbohydrates as well as disaccharides in the small intestine, leading to the suppression of α-glucosidase.
of meal-induced increase of plasma glucose. Hence, it may useful in the management of T2D. Based on the lead fractions obtained from in-vitro studies, we are going to plan an in-vivo study for further confirmation of the obtained results.

Limitations of the Study

Active compounds isolation from the fractions and its structural elucidation by nuclear magnetic resonance spectroscopy may helpful to develop newer antidiabetic agents. Here our target was an in-vitro evaluation of α-amylase and α-glucosidase inhibitory activity of the hydro-alcoholic extract, and fractions of A. caudatum and C. argentea.

Conclusion

The results of the present study prove that the fraction 2 of the A. caudatum and fraction 4 of the C. argentea are effective α-amylase and α-glucosidase inhibitors, which may helpful to reduce the postprandial glucose levels. However, the principle compounds responsible for the inhibitory action of α-amylase and α-glucosidase need to be further identified and characterized. This may be useful for the development of new antidiabetic agents from native plant resources.

Acknowledgments

Authors are thankful to KLE University and Principal, KLE University’s College of Pharmacy, Belagavi for providing the necessary facilities to carry out the work. We are thankful to VGST, Department of IT, BT, and SandT, Government Karnataka for financial support under VGST-CISE scheme. Authors are also thankful to Dr. Subarna Roy, Scientist D, RMRC (ICMR) Belgaum for the valuable support to our research work.

Financial Support and Sponsorship

Nil.

Conflicts of Interest

There are no conflicts of interest.

References

1. Mitra A, Dewanjee D, Dey B. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J Diabetes 2012;3:201-7.
2. Baron AD. Postprandial hyperglycaemia and alpha-glucosidase inhibitors. Diabetes Res Clin Pract 1998;40 Supp: S51-5.
3. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. Curr Sci 2002;83:30-8.
4. Ghadyale V, Takalkar S, Haldavnekar V, Arvindekar A. Effective control of postprandial glucose level through inhibition of intestinal alpha glucosidase by Cymbopogon martinii (Roxb.) Evid Based Complement Alternat Med 2012;2012:372909.
5. Singh SK, Rai PK, Jaiswal D, Watal G. Evidence-based critical evaluation of glycomic potential of Cynodon dactylon. Evid Based Complement Alternat Med 2007;8:415–20.
6. Vaidya BL. Some Controversial Drugs in Indian Medicine. Varanasi: Chaukhamba Orientalia; 1982. p. 86-8.
7. Sharma PV. Dravyaguna Vijnana. Vol. II. Varanasi: Chaukhamba Bharati Academy; 2005. p. 713.
8. Vaishyalar Shaligramaj. Shaligramanigantubhushanam. Vol. 7-8. Guduchyad varga. Bombay: Kemarar Shri Khirishadasa Prakashana; 2002. p. 361-2.
9. Nidavani RB, Mahalakhshmi AM, Seema M, Krishna KL. Pharmacology of Celosia argentea L. J Atoms Mol 2014;4:635-44.
10. Cos P, Villetinck AJ, Bergh EV, Maes L. Anti-infective potential of natural products: How to develop a stronger in-vitro proof-of-concept. J Ethnopharmacol 2006;106:230-302.
11. Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in-vitro. Exp Toxicol Pathol 2013;65:305-8.
12. Shai Li, Magano SR, Lebelo SL, Mogale AM. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. J Med Plant Res 2011;5:2863-67.
13. Hasani-Ranjbar S, Larijani B, Abdollah M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. Arch Med Sci 2008;4:285-92.
14. Mishra SB, Rao CV, Ojha SK. An analytical review of plants for anti diabetic activity with their Phytoconstituents and mechanism of action. Int J Pharm Sci Res 2010;1:1647-52.
15. Hara Y, Honda M. The inhibition of alpha amylase by tea polyphenols. Agric Biol Chem 1990;54:1939-45.
16. Matsui T, Tanaka T, Tamura S, Toshima A, Tamaya K, Miyata Y, et al. Alpha-glucosidase inhibitory profile of catechins and theaflavins. J Agric Food Chem 2007;55:99-105.
17. Saha D, Ghosh SK, Das T, Rahman H. Effect of Adiantum caudatumin streptozotocin-induceddiabetes mellitus in rats. Int Res J Pharm Appl Sci 2011;1:9-15.
18. Ghule S, Prakash T, Kotresha D, Karki R, Surendra V, Goli D. Anti-diabetic activity of Celosia argentea root in streptozotocin-induced diabetic rats. Int J Green Pharm 2013;9:6-11.
19. Behrens C, Ke CQ, Tang CP, Hohnen-Behrens C, et al. Anti-diabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chem Biol 2008;15:263-73.
20. Rohb J, Kroll J. Inhibitory effects of plant phenols on the activity of α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes; An update. Mini Rev Med Chem 2010;10:315-31.
21. Sales PM, Souza PM, Somei LA, Silveira D. A-amylase inhibitors: A review of raw material and isolated compounds from plant source. J Pharm Pharm Sci 2012;15:141-83.
22. Brahmacari G. Bio-flavonoids with promising antidiabetic potentials: A critical survey. In: Tiwari VK, Mishra BB, editors. Opportunity, Challenge and Scope of Natural Products in Medicinal Chemistry. Vol.2. Trivandrum: Research Signpost; 2012. p. 187-212.
23. Tan MJ, et al. Antidiabetic activities of triterpenoids isolated from bitter melon associated with activation of the AMPK pathway. Chem Biol 2008;15:263-73.
24. Rohn S, Rawel HM, et al. Inhibitory effects of plant phenols on the activity of selected enzymes. J Agric Food Chem 2002;50:3566-71.
25. Shimizu M, Kobayashi Y, Suzuki M, Satsu, Miyamoto Y. Regulation of intestinal glucose transport by tea catechins. Biofactors 2010;1:631-5.
26. Kim J, Kwon CS, Son KH. Inhibition of alpha-glucosidase and amylase by luteolin, a flavonoid. Biosci Biotechnol Biochem 2000;64:2458-61.