Screening of leaf extract of *Zingiber officinale* for hypoglycemic activity

Ambily PG[1], Jane Mathew[1]*,Sudhina M[2]

[1*]Jane Mathew, Associate professor, NGSM Institute of Pharmaceutical sciences, Nitte (Deemed to be University), Paneer, Deralakatte-575018,Karnataka State

[2] Yenepoya college of pharmacy & research centre, University Road, Deralakatte Mangalore, Karnataka -575018

Keywords: Ginger leaves; methanolic; aqueous extract,invitro antidiabetic activity

INTRODUCTION

Diabetes Mellitus is known ironically 'a disease of rich man'. Diabetes is a polygenic condition, with impaired metabolism of carbohydrates, fats and proteins this cause serious damage to heart, blood vessels, eyes, kidney and nerves [1]. According to International Diabetes Federation, 463 million adults from the age group of 20 – 79 yrs are affected with diabetes in worldwide and 4.2 million death cases were reported. In India 62 million, which is more than 7.2% of adult population are living with diabetes; which makes second most affected in world after China [2]. Management of hyperglycemia is to inhibit the metabolism of carbohydrates by enzymes in digestive tract; this will lower the blood glucose level in body [3]. Since the cases are increasing in both developing as well as developed countries in an alarming rate, it become a necessity to have an alternative and more effectual hyperglycemic drugs and their systematic studies [4]. Number of therapeutic agents such sulfonylureas, thiazolidiones, α-glucosidase inhibitors- Miglitol, Voglibose and Acarbose are widely accepted drugs to manage hyperglycemia. Many of these drugs have side effects as well as high cost with limited success[5]. According to WHO, 80% of population in developing countries utilize plants as their traditional medicines due to cost effective management and fewer side effects [6].

Ginger (Zingiber officinale) is a perennial herbaceous plant used in world wide for various medical ailments and as a spice over 2000 years, belongs to Zingiberaceae family. William Roscoe (1753-1831), an English botanist has given the name zingiber, a word derived from 'singabera' means horn-shaped with protrusions on the rhizome. Ginger is known other names like Cochin ginger, African ginger GanJiang, Jamaican ginger, Black ginger, Gegibre, Ingwer, and Race ginger. Its arial parts will grow up to three feet in height, its stalk will stick up to about 12 inches. Stalks are covered with two ranked leaves. Flower buds are seen as clusters of white and pink and yellow colour when it blooms. Rhizomes are pale buff colour with pungent taste and agreeable aromatic odour. Ginger contains 3% essential oil, 5-8% of pungent resinous mass and starch. The major active principles are as zingerone, gingerdiol, zingibrene,

gingerols and shogaols. Aroma and pungency is due to the presence of key components such as volatile essential oil and non volatile pungent compound oleoresin. Ginger is known for many medicinal properties such as cardioprotective, anti-inflammatory, antimicrobial, antioxidant, antiulcer, anticlotting and anticancer properties etc. The ginger is also used as growth promoter and an immunostimulant.[7,8,9]

The need for new hypoglycemic drug that does not weigh heavily on the pockets but proves to be efficacious has become a necessity in order to control the diabetic cases globally. Though Ginger rhizomes are commonly used, the leaves of the herb are not so popular . the medicinal value of a plant is based on the presence of bioactive constituents present in the plant. Advance phytochemical analysis can be done with the help of various chromatographic techniques like HPLC,LC-MS and GC-MS[10]. LC-MS/MS is a chromatographic technique to obtain structural information in synthetic chemistry, to analyze and determine the structure of degradants in active pharmaceutical ingredients and is also used to get information about the chemical structure of secondary plant metabolites[11]. In the present study based on the preliminary phytochemical studies which showed the presence of flavonoids, phenolic compounds, saponins and tannins that led to the testing of ginger leaf extract for in-vitro anti-diabetic activity.

MATERIALS AND METHODS

Collection of plant material

The leaves of Zingiber officinale were collected in and around Thrissur Kerala, in the month of June - August 2020. The collected plant was authenticated by Dr. Raju Krishna Chalannavar Professor and Chairman, Department of Applied Botany, Mangalore University, Mangalagangothri - 574 199.

Preparation of extract

The collected leaves were cleaned; shade dried and coarsely powdered using electrical blender. The powder was macerated with methanol for 7 days and extract was filtered through muslin cloth. The extract was concentrated by evaporation under controlled temperature (45-500C) and pressure (55 PSIg) using Rotavap Rotary evaporator (model: PBU-6D).

Preliminary phytochemical analysis

The phytochemical analysis of the extracts was performed as per the standard procedures described by Trease and Evans [12,13]. The extract was evaluated for Alkaloids, Flavonoids, Triterpenoids/ steroids, Glycosides, Carbohydrates, Saponins, Phenolic compounds and Tannins.

Screening of invitro anti-diabetic activity

Alpha Amylase-Inhibition Assay[14]:

The reaction mixture for assay is prepared by 200µL sodium phosphate buffer (0.02M) containing 20µL of α-amylase solution (in phosphate buffer 0.5µg/ml) and varing concentration of 200µL leaf extracts (10-100µg/ml). Mixture was incubated at 25°C for about 10 minutes. After the incubation, 200µl of 1% starch solution in 0.02M sodium phosphate

buffer at pH 6.9 is added to each test tubes. The reaction is stopped by adding 400µl of di-nitrosalicylic acid colour reagent. The test tubes are kept for incubation in boiling water bath for 5 minutes, and then it is cooled in room temperature and diluted with 15ml of distilled water. The absorbance of mixture is recorded at 540nm. Acarbose is included as a standard at various concentrations (10 - 100µg/ml). Without test substance is set up in parallel as a control and each experiment is performed in thrice. Percentage inhibition is calculated using the following formula,

Inhibitory activity (%) = (Ac −As/Ac) ×100

Where, As = test substance absorbance

Ac= absorbance of control.

2. Alpha glucosidase inhibition assay[15]:

Reaction mixture is prepared using 50µl phosphate buffer (100mM, pH-6.8), 10µl α-glucosidase(1U/ml), and 20µl of different concentrations of extract and fractions (10-100µg/ml) is poured into the 96-well plate pre-incubated for about 15 min at 37°C. To the above mixture, add 20µl p-nitro phenyl-α-D-glucopyranoside solution (5 Mm) as a substrate and incubated again for 15 min at 37°C. 50µl sodium carbonate (Na_2CO_3) (0.1M) is added to cease the reaction. By using microplate reader note down the absorbance of released p-nitrophenol at 405 nm. At various concentrations of Acarbose (10 -100µg/ml) is taken as a standard. Solution without test substance is taken as a control and each experiment must be repeated thrice. The percentage inhibition can be calculated using following formula,

Inhibitory activity (%) = (Ac − As/Ac)×100

Where, As = test substance absorbance

Ac = control absorbance.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis was carried out in methanolic and aqueous extract

Methanolic extract showed the presence of Flavonoids, Triterpenoids, glycosides, Saponins, phenolic compounds and tannins and aqueous extract revealed the existence of Flavonoids, Saponins, phenolic compounds and tannins and is shown in Table 1.

Table 1: results of preliminary phytochemical analysis of extract of Zingiber officinale.

Phytochemical constituents	**Inference**	
	Methanolic	**Aqueous**
Alkaloids	-	-
Flavonoids	+	+
Triterpenoids / Steroids	+	-
Glycosides	+	-
Carbohydrates	-	-

Saponins	+	+
Phenolic compounds	+	+
Tannins	+	+

+ = positive, - = negative

Invitro anti-diabetic activity

The results of alpha amylase & alpha glucosidase invitro assay is shown in table 2 & 3 and figure 1&2 respectively.

a. Alpha- amylase inhibition assay:

Table 2: Effect of aqueous and methanolic Zingiber officinale leaf extracts on Alpha-amylase inhibition:

Tested material	Concentration(µg/ml)	% Inhibition of Alpha-amylase ± SEM	IC_{50} value
Acarbose	10 20 40 60 80 100	17.37±0.75 21.65±0.37 28.20±0.85 42.30±0.42 51.99±0.62 67.37±0.79	72.83
Methanolic extract of Zingiber officinale	10 20 40 60 80 100	4.85±0.93 9.71±1.23 18.21±1.01 27.12±0.84 36.16±0.82 43.72±1.30	112.28
Aqueous extracts of Zingiber officinale	10 20 40 60 80 100	8.63±1.99 16.19±1.01 21.86±1.23 31.03±0.97 37.78±1.17 46.96±0.84	101.87

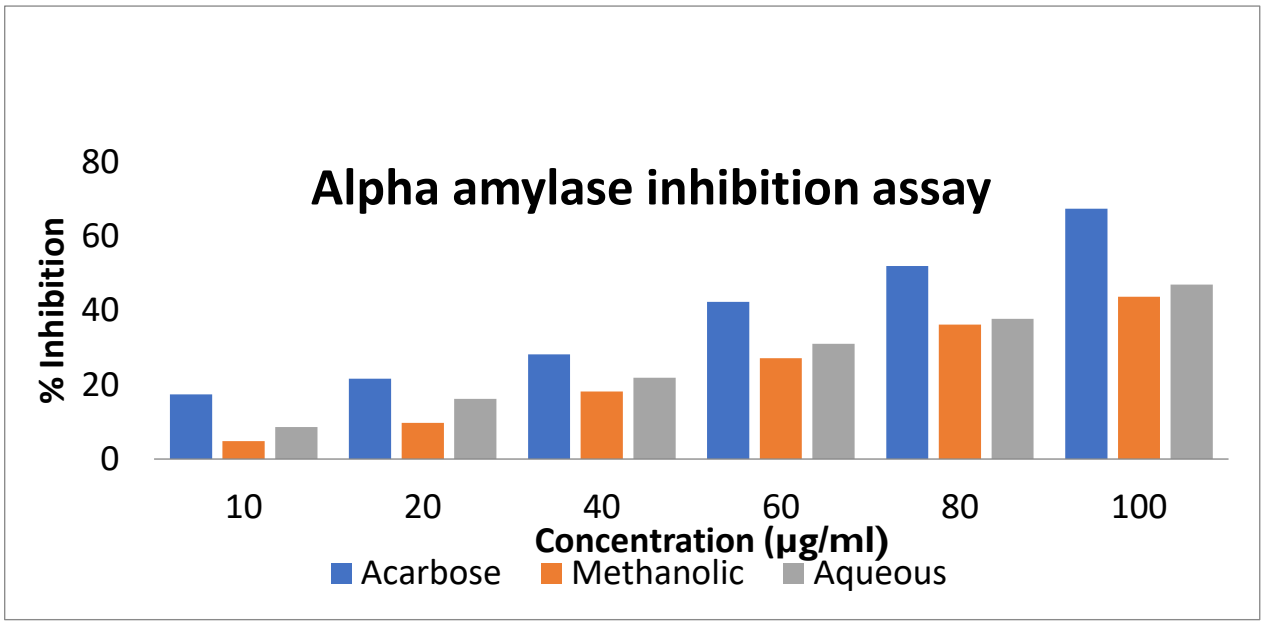

Figure 1: All values are expressed in terms of ± SEM and are found to be significant when compared to control P<0.05

Alpha-glucosidase inhibition assay:

Table 2: Effect of aqueous and methanolic Zingiber officinale leaves extracts on Alpha-glucosidase inhibition:

Tested material	**Concentration(µg/ml)**	**% Inhibition of Alpha-glucosidase ± SEM**	**IC_{50} value**
Acarbose	10 20 40 60 80 100	14.61±1.48 21.43±1.11 36.30±1.13 44.17±0.52 58.15±0.98 83.61±1.46	62.49
Methanolic extract of Zingiber officinale	10 20 40 60 80 100	19.47±0.41 24.48 ± 0.51 31.42 ± 0.40 36.11 ± 0.47 48.34 ± 0.47 70.02 ± 0.49	73.70
Aqueous extracts of Zingiber officinale	10 20 40 60 80 100	18.78±0.20 20.63±0.55 27.59±0.55 36.11±0.40 48.52±0.53 58.49±0.25	81.30

All values are expressed in terms of ± SEM and are found to be significant when compared to control P<0.05

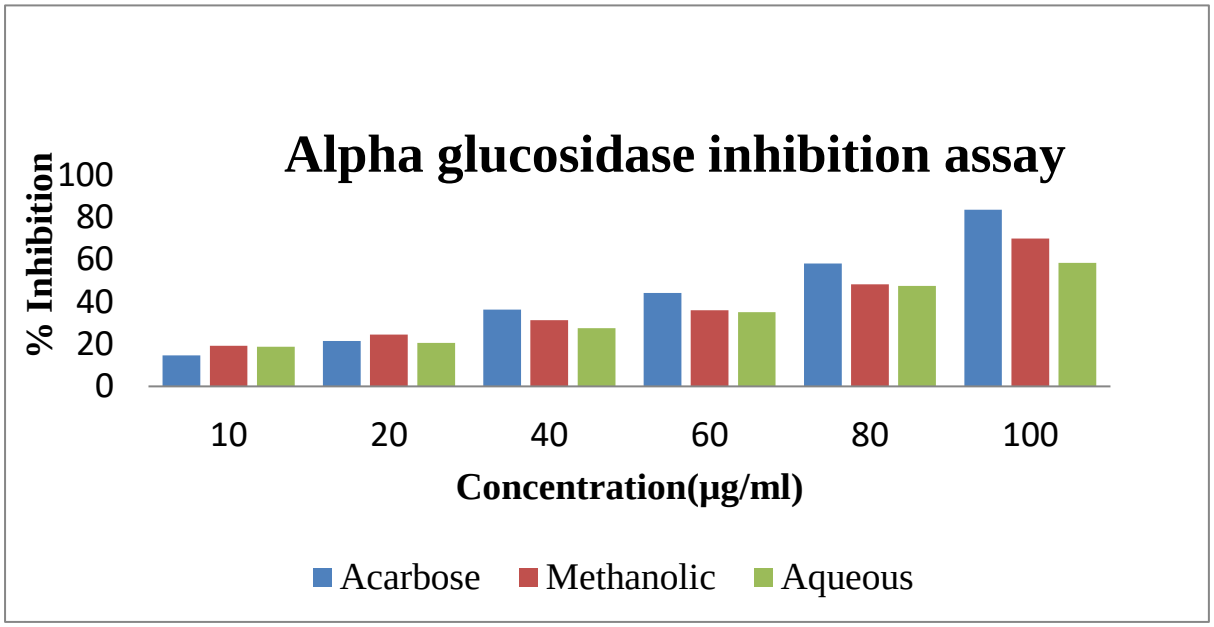

Figure 2: Alpha-glucosidase inhibition assay

DISCUSSION

Alpha – amylase inhibition assay results have approved the moderate anti diabetic activity in the both extracts. The standard drug Acarbose has showed the maximum % inhibition of 67.37% at a concentration of 100µg/mL whereas % inhibition of a concentration of 100µg/mL aqueous extract was seen at 46.96%. The methanolic extract showed maximum % inhibition at 100µg/mL a value of 43.72%. The IC50 values of methanolic and aqueous extracts were 112.28 and 101.87 respectively.

Alpha-glucosidase inhibition assay is based on the inhibition of the enzyme alpha – glucosidase and conformed the activity. The maximum inhibition activity was exhibited by methanolic extract as compared to aqueous at concentration 100µg/mL with percentage inhibition of 70.02%. The IC50 values of methanolic and aqueous extracts were 73.70 and 81.30% respectively. Concentration required for 50% inhibition of standard Acarbose was found to be 62.49µg/mL.

CONCLUSION

In the present study conducted in both aqueous and methanolic extracts showed moderate antidiabetic activity and this activity might due to the presence of phytoconstituents present in both extracts such as flavonoids, phenolic compounds saponins and tannins.

It can be concluded that, Zingiber officinale extracts had a moderate inhibitory effect on α-amylase and α-glucosidase enzymes in dose depended manner. Aqueous extract exhibits more α-amylase inhibition activity as compared to methanolic extract with highest percentage inhibition of 46.96±0.84%. Methanolic extract showed high percentage inhibition of 48.34 ± 0.47% than aqueous extract, when compared to Acarbose standard.

REFERENCES:

1. Reddy PH. Can Diabetes Be Controlled by Lifestyle Activities, Current research in diabetes & obesity journal. 2017 Mar;1(4).
2. SLDV RM, Das MC, Vijayaraghavan R, Shanmukha I. In vitro evaluation of antidiabetic activity of aqueous and ethanolic leaves extracts of Chloroxylon swietenia. National Journal of Physiology, Pharmacy and Pharmacology. 2017;7(5):486.
3. Ali H, Houghton PJ, Soumyanath A. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthus amarus. Journal of ethnopharmacology. 2006 Oct 11;107(3):449-55.
4. Sagbo IJ, van de Venter M, Koekemoer T, Bradley G. In vitro antidiabetic activity and mechanism of action of Brachylaena elliptica (Thunb.) DC. Evidence-Based Complementary and Alternative Medicine. 2018 Jan 1;2018.
5. Somtimuang C, Olatunji OJ, Ovatlarnporn C. Evaluation of in vitro α-amylase and α-glucosidase inhibitory potentials of 14 medicinal plants constituted in Thai folk antidiabetic formularies. Chemistry & biodiversity. 2018 Apr;15(4):e1800025.
6. Osabor VN, Bassey FI, Umoh UU. Phytochemical screening and quantitative evaluation of nutritional values of Zingiber officinale (Ginger). Chemical Science International Journal. 2015 Jul 10:1-6.
7. Khan MA, Ansair MI, Maheshwari RK. Phytochemistry and Pharmacological Properties of Ginger (Zingiber officinale)2018.
8. Bhatt N, Waly MI, Essa MM, Ali A. Ginger: A functional herb. Food as Medicine. 2013:51-71.
9. Jennifer Fernandes ,Sangeetha B, Ronald Fernandes , Nishmitha Gretta D'Souza,Invivo Anti-inflammatory Activity Potential of Ethanolic extract of Stem Bark of Sapindus trifoliatus Linn, Journal of Xi'an Shiyou University, Natural Science Edition,17(10)42-48.
10. Anjoo Kamboj.(2012),Analytical Evaluation of Herbal Drugs; Drug Discovery Research in Pharmacognosy,pg23-58
11. Jane M, Neethu MV. Detection of potential degradants in Oseltamivir by liquid chromatography-Tandem Mass Spectroscopy; Journal of Xi'an Shiyou University, Natural Science Edition 17(10):102-112.
12. Trease, G.E. & Evans, W. C. (1989). Trease and Evan's Textbook of Pharmacognosy. 13th Edition. Cambridge University Press, London. 546.
13. Gul R, Jan SU, Faridullah S, Sherani S, Jahan N. Preliminary phytochemical screening, quantitative analysis of alkaloids, and antioxidant activity of crude plant extracts from Ephedra intermedia indigenous to Balochistan. Sci. World J. 2017;201-7.
14. Banerjee A, Maji B, Mukherjee S, Chaudhuri K, Seal T. In Vitro Antidiabetic and Anti-oxidant Activities of Methanol Extract of Tinospora Sinensis. Journal of Applied Biology & Biotechnology Vol. 2017 May;5(03):061-7.
15. Andrade-Cetto A, Becerra-Jiménez J, Cárdenas-Vázquez R. Alfa glucosidase inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes. Journal of ethnopharmacology. 2008 Feb 28;116(1):27-32.