

Screening of *Kedrostis Foetidissima* (Jacq.) Cogn. Extracts for α -Amylase Inhibition

AMUTHA MUTHUSAMY¹, LALITHA POTTAI²

¹ Department of Chemistry, Arulmigu Palaniandavar Arts College For Women, Chinnakalayampthur, Palani-624615, Dindigul District, Tamilnadu, India

² Department of Chemistry, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamilnadu, India

Abstract: The α -amylase inhibition potential of extracts of *Kedrostis foetidissima*(Jacq.)cogn. was determined through Dinitro salicylic acid method. The IE50 of all eight extracts show excellent inhibition activity. IE50 of the extracts ranges from 21.02 μ g/ml (KFSEA) to 37.14 μ g/ml (KFSPE). Ethyl acetate and ethanol extract show excellent inhibition efficiency at 30 μ g/ml which is comparatively higher than that of the standard drug Acarbose revealing this plant to be a potent natural remedy for diabetic patients, without side effects.

Keywords: Antidiabetic, *Kedrostis foetidissima*, α -amylase.

1. INTRODUCTION

Diabetes mellitus, normally called as Diabetes, is a syndrome due to high blood sugar level for long period [1], which causes frequent urination and hunger [2]. It also leads to cardiovascular disease and Kidney failure when untreated [3]. Until 2014, it was reported that, worldwide, 387 million people have diabetes [4]. Almost 8.3% of adults were suffering from diabetes [5]. Reports reveal 592 million likely to suffer from diabetes by 2035 [6]. India currently has the prevalent figure of diabetic patients worldwide and designated as the ‘diabetic capital of the world [7]. In the past years, the use of herbal medicines have found more importance due to lack of complete remedy, cause of side effects and high drug cost in allopathic treatments.

Kedrostis foetidissima, *Cucurbitaceae* family medicinal plant, traditionally named as “Appakovai” in Tamil, is reported to be used to cure various ailments such as common cold [8], asthma [9], small pox [10], urinary tract infection [9], in treating snake bites [11], cattle bloat [12]. Extracts of *Kedrostis* was proved to be effective against Lung cancer –A549 [13] and Breast cancer - MCF7 & YMB1 [14] cell lines. This plant shows appreciable antioxidant and reducing property [15].

Pancreatic juice and saliva contains α - amylase, a prominent digestive enzyme, responsible for the conversion of insoluble starch to soluble compounds [16]. Inhibition of α - amylase, reduces the rate of conversion of carbohydrates to absorbable compounds, which in turn reduces the discharge of blood glucose after meal [17]. Diminished levels of postprandial hyperglycemia were effectively achieved by α -amylase inhibitors [18]. *Aspergillus niger* shows excellent yield of α -amylase on solid state fermentation [19] and works for a wide range of pH also active at the temperature of 60°C for even 1hr [20]. So our present study was aimed to screen various extracts of *Kedrostis* towards its inhibition activity on α -amylase, isolated from *Aspergillus niger*, may be a facile screening tool for antidiabetic activity.

2. METHODS

2.1. Collection of Plant Materials:

Kedrostis foetidissima was collected from Aliyar hills, near Pollachi. The voucher specimen submitted to Botanical Survey of India, Tamilnadu Agri. University, Coimbatore, Tamilnadu was identified as *Kedrostis foetidissima* (jacq.) cogn = *Trichosanthes foetidissima* Jacq.) *Cucurbitaceae* family by Dr.P.Satyanarayanan, Scientist-D (BSI/SRC/5/23/2010-11/Tech.-1309). The collected plant materials were washed thoroughly to remove mud particles, separated and then shade dried. The stem and leaves were crushed and stored in hermetically sealed containers for further use.

2.2. Extraction of Plant Materials:

The leaves and stem (100 g) of *Kedrostis foetidissima (jacq.) cogn.*, were first defatted with petroleum ether. Ethanol (ETOH), Chloroform (CF), Ethyl Acetate (EA), Petroleum Ether (PE) extract of this plant is prepared by refluxing (6h) 100g pulverized leaves and crushed stem with 1 litre of each solvent mentioned above. All extracts were concentrated to get dry residues. The dry extracts were refrigerated for future use.

2.3. α -Amylase Inhibition Activity:

The various extracts of *K.foetidissima* were analysed for its inhibition against the enzyme α -amylase, obtained from the fungi, *Aspergillus niger*. Standard procedure reported in literature was adopted in this study [21].

2.3.1. Preparation of solutions:

Stock solution was prepared by dissolving 1 g of various extracts in 10 ml DMSO. Test samples were prepared by taking 20 μ l of the stock solution and diluted to 2ml. This solution was further diluted to four different concentrations such as 10 μ g/ml, 20 μ g/ml, 30 μ g/ml and 40 μ g/ml in 1.0ml each. Starch solution (0.1% w/v) was prepared by mixing 0.1g starch powder in 100 ml 16mM Sodium Acetate buffer (pH 6.9). Enzyme solution was obtained by stirring 27.5mg α -amylase in 100ml purified water. Colorimetric reagent was prepared by mixing 3,5-Dinitro salicylic acid (96mM) and sodium potassium tartrate. Blank was prepared by adopting similar mixing procedure without test sample. The results obtained were compared with the standard Acarbose.

2.3.2. *In vitro* inhibition of α -amylase:

Test samples of various extracts of different concentrations (10 μ g/ml, 20 μ g/ml, and 30 μ g/ml) were taken in separate test tubes with proper labelling. To the test samples 1ml starch solution and 1ml DMSO were added and incubated at 25°C for 3min. The contents were mixed thoroughly with 1ml enzyme solution and incubated at 25°C for 3 minutes. The colour of the mixture was noted. To the above mixture 1ml colouring reagent was added to stop the reaction and heated in a water bath for 15 min. The contents were cooled and the absorbance was noted at 540nm using photo colorimeter. The test sample reacts with the enzyme and leads to the formation of Maltose through the reduction of 3,5-Dinitrosalicylic acid. Percentage inhibition efficiency (IE%) was calculated using the equation (1).

$$IE \% = (A_b - A_s) / A_b \times 100 \quad (1)$$

where, A_b is the absorbance of the blank and A_s is the absorbance of the sample.

3. RESULTS AND DISCUSSION

Eight different solvent extracts of *K.foetidissima* at various concentrations (10 μ g/ml, 20 μ g/ml and 30 μ g/ml) were analysed for its inhibition efficiency against the enzyme α - amylase and compared with the standard Acarbose. Table -1. Shows the inhibition efficiency of the standard Acarbose at 250 μ g/ml was 66%. The results obtained are presented in Table-2 and Table-3. Almost all the extracts shows appreciable inhibition efficiency towards the enzyme. Leaf and stem ethylacetate, leaf ethanol extracts shows 77.77 %, 72.22% and 72.22% inhibition at 30 μ g/ml respectively. Except two pet ether extracts all other six extracts shows more than 50% inhibition at 30 μ g/ml. At lower concentration (10 μ g/ml) the inhibition percentage was zero for leaf chloroform, stem pet ether and ethylacetate extracts.

Fig 1 and Fig 2. Shows the relation between the IE % vs concentration of leaf and stem extracts of *K.foetidissima*. With the help of scattered plot, the equation of the straight line, R^2 value and IE50 values were obtained for all the extracts.

IE50 values disclose all the extracts shows excellent results towards the inhibition of α - amylase enzyme. Stem and leaf Ethyl acetate extracts were more effective with IE50 values of KFSEA-21.02 μ g/ml and KFLEA-23.11 μ g/ml. Leaf and stem ethanol extracts also have IE50 values as KFLEOH -24.19 μ g/ml and KFSEOH- 26.67 μ g/ml, IE50 value of Stem Chloroform extract is 25.98 μ g/ml, where as that of leaf is 29.63 μ g/ml. This shows the plant is a best source of antidiabetic drug and the presence of compounds responsible for this inhibition need to be identified.

Table -1. Inhibition Efficiency of Standard Acarbose

Sample		Conc (μ g)	% IE
Acarbose	Positive control	250 μ g/ml	66.66

Table-2. Inhibition Efficiency of Leaf Extracts of *K.Foetidissima*

Conc (µg/ml)	Sample code			
	KFLPE	KFLCF	KFLEA	KFLEOH
	IE %	IE %	IE %	IE %
10	15.38	0	15.38	23.07
20	38.88	14.28	27.77	23.81
30	38.88	55.55	77.77	72.22

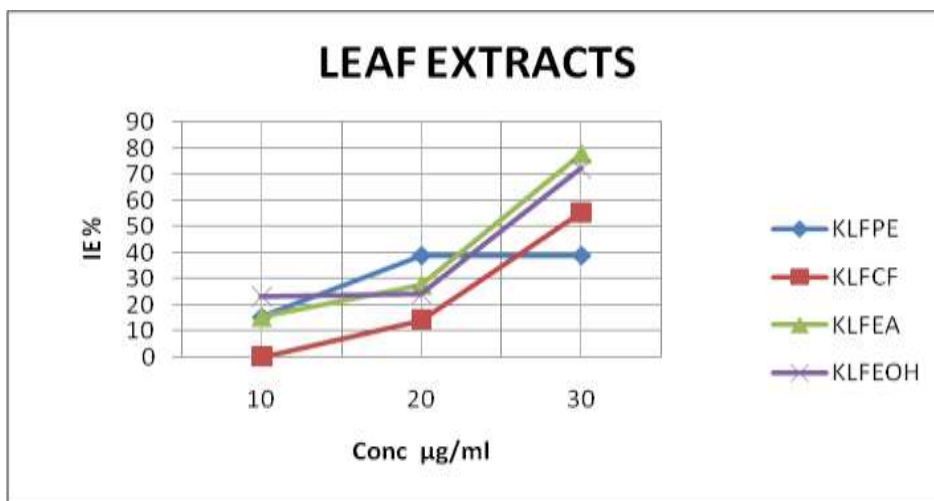


Fig-1. IE % Vs Conc of Leaf Extracts of *K.Foetidissima*

Table - 3. Inhibition Efficiency of Stem Extracts of *K.Foetidissima*

Conc (µg/ml)	Sample code			
	KFSPE	KFSCF	KFSEA	KFSEOH
	IE %	IE %	IE %	IE %
10	0	7.7	0	15.38
20	11.11	33.33	66.66	16.66
30	38.88	61.11	72.22	66.66

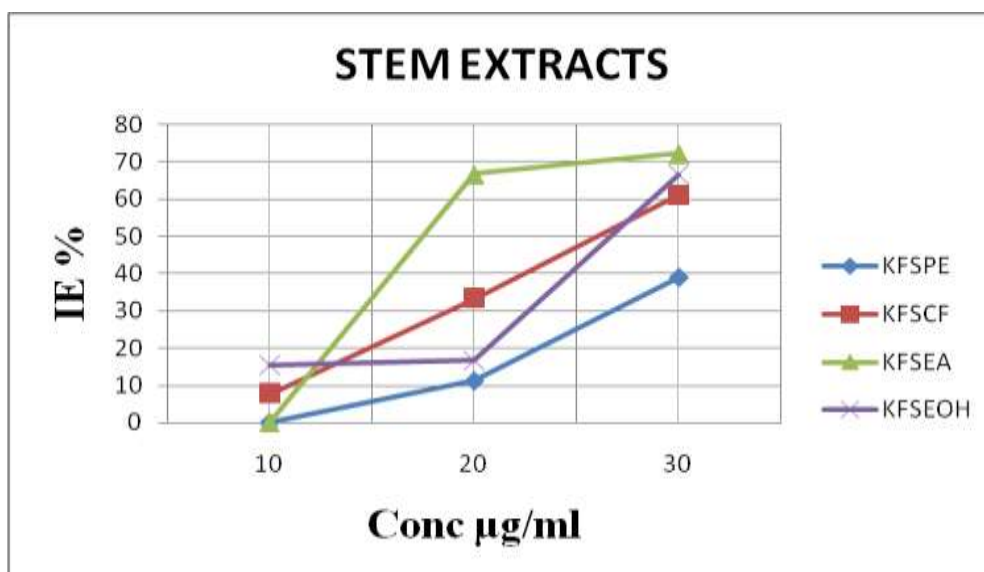


Fig-2. IE % Vs Conc of Stem Extracts of *K.Foetidissima*

Table - 4. R² and IE50 values of various extracts

S.No	Sample code	Equation	R ²	IE 50 (µg/ml)
1.	KFLPE	$y = 1.175x + 7.546$	0.750	36.13
2.	KFLCF	$y = 2.777x - 32.27$	0.927	29.63
3.	KFLEA	$y = 3.119x - 22.08$	0.892	23.11
4.	KFLEOH	$y = 2.457x - 9.45$	0.761	24.19
5.	KFSPE	$y = 1.944x - 22.21$	0.942	37.14
6.	KFSCF	$y = 2.670x - 19.36$	0.999	25.98
7.	KFSEA	$y = 3.611x - 25.92$	0.807	21.02
8.	KFSEOH	$y = 2.564x - 18.38$	0.768	26.67

Kedrostis foetidissima is a traditional medicinal plant, can be easily grown in home, and has extensive medicinal applications. Various extracts of this plant shows prominent antioxidant, antibacterial, antifungal activities. Moreover this plant is effective in healing wounds. Reports also reveal ethanol and methanol extracts of KF, effectively hinders the growth of Breast cancer (MCF-7) and Human Lung cancer (A-549) cells and possess anticancer activity. Literature search discloses sporadic reports on the antidiabetic activity on this species. Antidiabetic activity of seed and leaf of *Kedrostis* was reported for three extracts each [22], our present study was aimed to evaluate antidiabetic activity of stem and compared with leaf extracts by calculating IE50 values for each extract.

Results of the present assay values reveals this plant is a potent herbal source for antidiabetic drug. It may act as a potent inhibitor against the enzyme α - amylase. Generally inhibitors reduce the rate of hydrolysis of polysaccharides such as starch and glycogen producing glucose and maltose, which in turn decrease the blood sugar. The standard drug, Acarbose used now-a-days for treating type-2 diabetics is reported to cause allergy, inflammatory bowel and stomach disorders, kidney trouble and diabetic ketoacidosis. *Kedrostis* may be recommended as an inhibitor without any side effects. The study also assures α -amylase assay to be a facile screening tool in determining the antidiabetic potential of plant extracts. This multiple medicinal efficiency of this plant indended us in the isolation of metabolites through column chromatography, which is in progress.

4. CONCLUSION

India is “Diabetic capital of the world”. Among 171 million patients around the world, 40 million are from India. It is time to identify effective and potent herbal based drugs with no side effects for treating diabetics. The findings of the present study affirms *Kedrostis* to be a potent antidiabetic herbal based drug which inhibits the activity of α - amylase without any side effects. Isolation of compounds that are responsible for the antidiabetic efficacy is needed.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the concerned authorities of the Avinashilingam Institute for Home Science and Higher Education for Women University, Coimbatore, for providing all possible facilities to carry out the research work effectively.

REFERENCES

- [1] Diabetes Blue Circle Symbol. International Diabetes Federation. 17 March, 2006.
- [2] About diabetes. World Health Organization. Retrieved 4 April, 2014.
- [3] Diabetes Fact sheet N°312. WHO. October 2013. Retrieved 25 March, 2014.
- [4] Update (2014) IDF. International Diabetes Federation.
- [5] Shi, Yuankai Hu, Frank B. (2014), The global implications of diabetes and cancer, *The Lancet*, p.383.
- [6] Picot J, Jones J, Colquitt JL, Gospodarevskaya E, Loveman E, Baxter L, Clegg AJ(2009) The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. *Health technology assessment (Winchester, England)* 13(41):1–190, 215–357.

- [7] Abate N and Chandalia M.(2007) Indian Journal of Medical Research,125:251-258.
- [8] Karuppusamy S(2007) Medicinal plants used by Palian tribes of Sirumalai hills of southern India. Natural product radiance,6(5):436-442.
- [9] Giday M(2001) An Ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. Skriftserie, 3:81-99.
- [10] Tabuti JRS, Lye KA, Dhillion SS(2003)Traditional herbal drugs of Bulamogi, Uganda: Plants, use and administration. J. Ethnopharmacol,88:19-44.
- [11] Dymock W (1981) Pharmacogeophia Indica. Bischen Singh Mahendra Pal Singh, Dehradun, Periodical experts. New Delhi 2:90-92.
- [12] Ole-Miaron JO(2003)The Maasai ethnodiagnostic skill of livestock diseases: a lead to traditional bioprospecting. Journal of Ethnopharmacology 84:79 - 83.
- [13] Amutha M and Lalitha P(2015) Biological Effect of *Kedrostis foetidissima* on A-549 Lung Cancer Cell Lines. International J Ext Res.5:10-13.
- [14] Choene M, Motadi LR(2012) Anti-Proliferative Effects of the Methanolic Extract of *Kedrostis foetidissima* in Breast Cancer Cell Lines. Mol Biol. 1(1):107.
- [15] Amutha M and Lalitha P (2015) Evaluation of anti oxidant activities of stem and leaf extracts of *Kedrostis foetidissima Jacq*) Cogn. Int J Res Pharm Sci.5(1):28 – 34.
- [16] Afifi AF, Kamel EM, Khalil AA, Foad MA, Fawziand EM and Houseny H(2008) Purification and characterization of α -amylase from *Penicillium olsonii* under the effect of some antioxidant vitamins. Global Journal of Biotechnology and Biochemistry 3(1):14–12.
- [17] Kwon YI, Apostolidis E and Shetty K(2007) Evaluation of Pepper (*Capsicum annum*) for management of diabetes and hypertension. Journal of Food Biochemistry 31(3): 370–385.
- [18] Matsui T, Ogunwande TA, Abesundara KJM, Matsumoto K (2006)Anti-hyperglycemic potential of natural products. Mini-Reviews in Medicinal Chemistry,6(3): 349–356.
- [19] Kumar A, Duhan JS(2011) Production and Characterization of Amylase Enzyme Isolated from *Aspergillus niger* MTCC-104 Employing Solid State Fermentation. Int. J. Pharma and Bio Sciences 2(3):250-258.
- [20] Jahir Alam Khan and Sachin Kumar Yadav(2011) Production of Alpha Amylases By *Aspergillus Niger* Using Cheaper Substrates Employing Solid State Fermentation.International Journal of Plant, Animal and Environmental Sciences, 1(3):100-108.
- [21] Worthington V(1993) Worthington Enzyme Manual Freehold, Worthington Bio-chemical Corporation, New Jersey, pp.36–261.
- [22] Nirmala J and Pandian R (2015) Study of anti-diabetic activity of *Kedrostis feoditissima (jacq.)Cogn.*, Int.J. Curr. Res. Aca. Rev 3(7):50-55.