



RESEARCH ARTICLE

ALPHA AMYLASE INHIBITION ACTIVITY OF OKRA MUCILAGE.

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Abstract

In the present study the aqueous seed extract of okra Mucilage was used to examined for alpha amylase inhibition activity. Different concentrations of extracts were incubated with enzyme substrate solution and the activity of enzyme was measured. The maximum inhibition activity was observed at 240 μ l concentration. The starch agar plate method was also prepared for detection of alpha amylase inhibitor. This inhibitory compound also has insecticidal activity. Diabetes Mellitus is a metabolic disorder characterized by high blood glucose level caused due to deficiency of insulin action. The inhibition of carbohydrate hydrolyzing enzyme such as alpha-amylase can be important strategy in blood glucose level in patients with type II diabetes. In this study male mice were used to measure blood glucose level. The results show that the anti-amylase inhibitor was very powerful to reduce the level of glucose in the blood of the treated mice to the normal comparison with the control. The inhibitory compound was analyzed by FT-IR and SDS-PAGE.

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Introduction:-

Okra Mucilage is an important vegetable. This plant is cultivated in tropical, subtropical and warm temperate region, which is widely distributed from Africa to Asia, Southern Europe and America. This Okra Mucilage plant belongs to the family Malvaceae, botanical name of this plant is *Abelmoschus esculentus*. Okra Mucilage plant plays an important role in the human diet by supplying carbohydrates, minerals and vitamins, potassium, sodium, magnesium were found to be the principal element. Okra is a power house of valuable nutrients including fiber, vitamin B₆ and folic acid. Okra is rich in fiber, both soluble and insoluble. Studies conducted at universities of Wisconsin, Madison, USA has revealed that soluble fiber present in Okra helps to lower serum cholesterol, reducing the risk of heart diseases and helps to stabilize blood sugar and binds cholesterol and bile carrying toxic wastes. Whereas, insoluble fiber helps to keep the intestinal tract healthy. Okra is known by many local names in different parts of world. It is called Lady's finger in the England 'Gumbo' in United State of America and 'Bhindi' in India (Chauhan et.al. 1972).

There are many enzymes in the human digestive system that help in the digestion of food. Alpha amylase catalyses the breakdown of polysaccharide into monosaccharide and only monosaccharide form of food only can absorbed in the stomach. Alpha amylase is an important enzyme that plays an important role in the digestion of starch and glycogen which is present in the saliva and in pancreas of mammals. Alpha amylase inhibitor which inhibit starch hydrolysis which is an important strategy in blood glucose level of Diabetes mellitus. Diabetes mellitus is a

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metabolic disorder characterized by high blood glucose level caused due to deficiency of Insulin action. Okra is said to be very useful as alpha amylase inhibitor (Greebbs et.al.,1977).

Materials and Methods:-

Collection and identification of plant material:-

Fresh Okra Mucilage (*Abelmoschus esculentus*) pod was collected from village Pokhari taluka Nandgaon district Nasik .The peel and seed were separated washed, and dried under shade and powdered.

Aqueous Extraction:-

Dry powder of Okra seeds were allowed for soxhlet's extraction in sterile distilled water in which 50 gm powder and 100ml distilled water were added and extract were collected in bottle. The bottle was allowed to cool and then filtered. The filtrate was used as aqueous plant extract.

The protein content in the extract was quantified following the method of Lowry et.al (1951).

Assay of Alpha Amylase Inhibitor activity:-

The 1% starch solution was obtained by stirring and boiling 1gm starch in 100 ml distilled water for 15min. 0.1% alpha amylase was prepared by mixing 0.1gm alpha amylase in 100ml of 0.2M Phosphate buffer (pH-7.0). Alpha amylase was premixed with the aqueous extract of okra seeds at various concentration from 20 to 250ul and kept it for 30 min. then this was added to 1ml starch. This was carried out at 37°C for 10 min. and the reaction was terminated by addition of 2ml DNSA (3, 5 dinitrosalicylic acid reagent). The reaction mixture was heated for 15min at 100°C and which is diluted with 10ml of D/W. This alpha amylase activity was determined by measuring absorbance at 540nm on spectrophotometer. Simultaneously blank control was also prepared by without Okra seed extracts.

Starch Agar Plate method for detection of Alpha Amylase Inhibition:-

Detection of alpha amylase inhibitor from okra seed extracts was done by the method described by (Possum and Whitaker 1974) with slight modification. The starch agar plates were prepared by adding 1% starch, 2% agar and 1.3% nutrient broth. Bacterial culture of *Bacillus amyloliquefaciens* was also spread on starch agar plates. α -Amylase and okra seed extract were incubated for 30 min. at 37°C and loaded in well of 6mm diameters from 20 to 240µl made in the starch agar plates these plates were incubated at 37°C for 24 hrs. Next day Plates were flooded with iodine solution and the excess solution poured off.

Pesticidal activity of Alpha Amylase inhibitor:-

Infected green chick peas plant was taken from which larvae (Yellow caterpillar) were collected in petri plate and 5ml aqueous extract of okra seed extract was flooded on larvae and kept it for 24hrs.

Alpha amylase inhibitor analysis by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide gel electrophoresis):-

Alpha amylase inhibitor was resolved by electrophoretically by SDS-PAGE. Preparing 12% resolving gel with tris buffer of pH-8.8 and 5% stacking gel with tris buffer of 6.8pH. And 20 µl sample was loaded and after electrophoretic run bands were visualized by staining with AgNO₃ (20%) (Sambrook and Russell method.) SDS-PAGE was done by using Bio Lab protein Marker (2-212 kDa).

FT-IR-analysis of alpha amylase inhibitor (Fourier transform infrared spectroscopy):-

Alpha amylase inhibitors was analyzed by FT-IR- instrument located in department of chemical technology Dr. Babasaheb Ambedkar Marathwada University Aurangabad, Maharashtra.

Amylase inhibitor treated mice (preliminary screening):-

First blood glucose level of diabetic mice were recorded which was 133/DL. The solution used in the treatment were prepared as follows.

One ml of aqueous seed extract of okra plant was dissolved in ten ml of distilled water it was then injected in mice for the first three days. After 24hrs. blood glucose level was checked.

Results:-

Assay of alpha amylase inhibition activity:-

The alpha amylase was assayed by quantifying the reducing sugar (maltose equivalent) liberated under the assay conditions. The enzyme inhibitory activity is expressed as the decrease in units of maltose liberated. A modified 3,5 dinitrosalicylic acid (DNSA) method of Bernfeld (1935) was followed to estimate the maltose content. From table no. 1 we can find that the percentage of inhibition is about 65% at 0.11 absorbance, and the maltose sugar liberated was only 70 μ g.

The percentage of inhibition of alpha amylase was calculated using the formula:

$$\text{Inhibition(\%)} = 100(\text{control-test})$$

Suitable reagent blank and inhibitor controls were simultaneously carried out and subtracted.

Table no. 1:- The activity of alpha amylase inhibitor from aqueous seed extract of Okra Mucilage plant.

Sr. no.	Standard O.D of alpha Amylase at 540 nm.	Alpha Amylase reducing Sugar con. in μ g (maltose)	Alpha Amylase Inhibitor O.D. at 540 nm.	Alpha Amylase Inhibitor reducing sugar con..in μ g (maltose)	percentage of inhibition of alpha amylase (%)
1.	0.23	200	0.43	398	24
2.	0.29	290	0.42	396	25
3.	0.32	320	0.38	300	30
4.	0.38	390	0.33	297	37
5.	0.47	450	0.29	228	42
6.	0.56	520	0.24	220	48
7.	0.65	600	0.23	210	48
8.	0.77	690	0.19	198	54
9.	0.85	800	0.16	100	58
10.	0.87	820	0.13	80	62
11.	0.93	860	0.11	70	65
12.	0.97	900	0.11	70	65

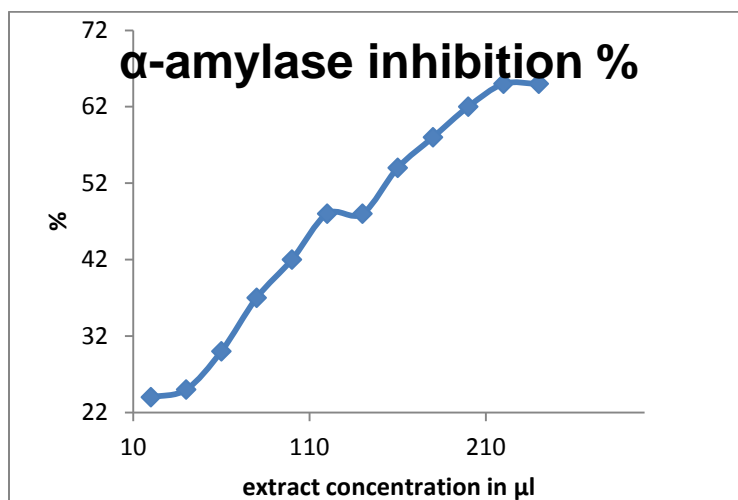


Fig.3:- Alpha-Amylase Inhibitor Percentage Graph. As the concentration of extract increases the percentage of inhibition also increases.

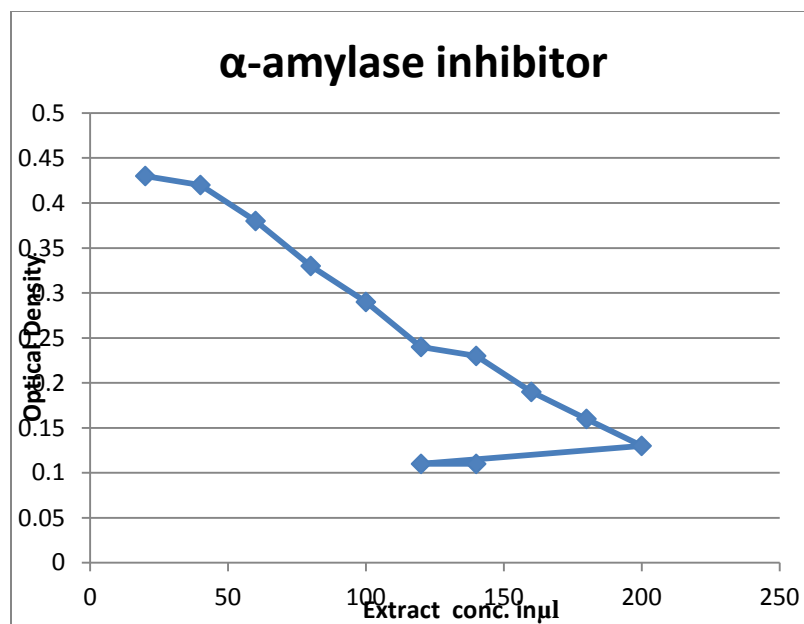


Fig 4:- Alpha amylase inhibitor concentration graph. As the Concentration of inhibitor increases the optical density decreases.

Starch agar plate detection of alpha amylase inhibition



Fig 5:- starch agar plate Starch agar plate figure five shows that in control well there is a clear zone of lysis of starch where as in alpha amylase inhibitor well there is no clear zone instead of zone there is a iodine color of starch is present.

Pesticidal activity of alpha amylase inhibitor:-

It was observed that alpha amylase inhibitor is a good pesticide because the larvae were totally killed within twenty four hours.

Sodium dodecyl polyacryl amide gel electrophoresis(SDS-PAGE)

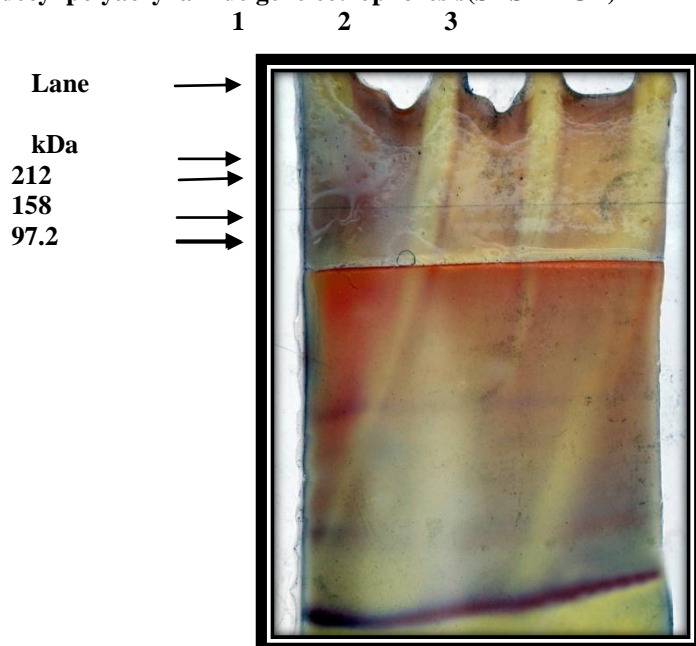


Fig.6:-Electrophoretic pattern of SDS-PAGE shows protein markers band in lane 1 and okra plant aqueous seed extract of alpha amylase inhibitor in lane 2 and 3, shows 212KD., 152KD and 116KD molecular weight band.

FT-IR-analysis of alpha amylase inhibitor (Fourier transform infrared spectroscopy):-

Alpha amylase inhibitor was analyzed by FT-IR- instrument located in the department of chemical technology Dr. Babasaheb Ambedkar Marathwada University Aurangabad, Maharashtra.

Observation **table 2**-Okra plant aqueous Seed extract.

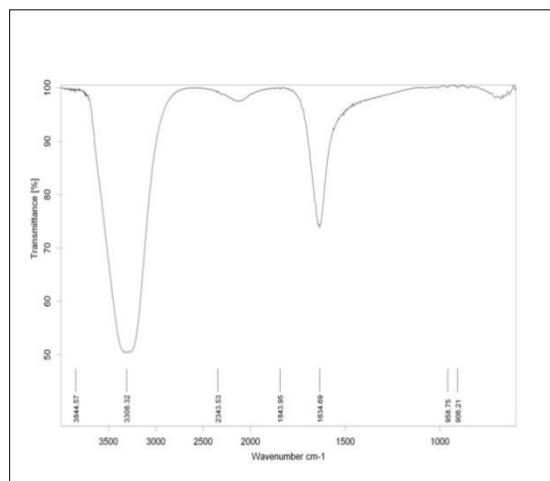


Fig 7:- FT-IR spectrum of aqueous extract of Okra seed.

Sr. no.	Wave no/cm ²	Functional group
1.	3288.04	H-bound
2.	2354.21	Alkynes
3.	1866.30	Anhydride
4.	1633.65	Amide
5	1555.98	Nitro (R-NO ₂)
6.	959.15	Aromatics (out of plane bend)
7.	906.33	Aromatics out of plane bend

Blood glucose level of treated mice:-

Blood glucose level in the treated mice were checked by *Arkray max glucocard 01ministrips* blood glucose test strips. It was found that blood glucose level of mice were decreased. Which was 75 mg/dl.

Discussion:-

In the present study alpha amylase inhibitor activity was determined using okra plant seed aqueous extract and inhibitory activity was confirmed by assay (DNSA) and starch agar plate method. This activity also analyzed by SDS-PAGE and FT-IR analysis further experiment can be performed on animal's models to confirm the hypoglycemic activity. The objective of our study is to investigate the hypoglycemic effect in the aqueous extracts of Okra seed.

By Sharma *et.al.*, (2006) medicinal plants tested showed strong inhibition of alpha amylase activity of diabetic and non-diabetic mice. Yoon and Robyt *et al.*, (2003) examined the inhibition kinetic of acarbose and its two analogues 4IV-maltohexosyl acarbose 4IV-maltododecaosyl acarbose on alpha amylase from four different sources *Aspergillus niger*, *Bacillus amyloliquefaciens* human saliva and porcine pancreas. The three inhibitors showed mixed noncompetitive inhibition for all four alpha amylase. A similar type of inhibition for *S.cumini* seed extract as evidenced by the Dixon plot was also obtained. By V.Sabitha *et.al.* (2006) alpha-glycosidase and alpha amylase inhibitory studies demonstrated that highest inhibitory activity at 250µg/ml. and our study demonstrated at 240µl/ml. Zohre Mirzaalian Dastjerdi *et al.*, (2015) reported that the alpha amylase inhibitor activity of the *Teucrium* species extract may be as a result of the existence of various phytochemicals.

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