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ENZYMATIC ACTIVITY OF MOMORDICA CHARANTIA FRUITS

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The crude enzyme fraction was isolated from the fresh unripe fruits of *Momordica charantia Linn* and screened for its amylolytic and lipolytic activity according to standard literatures. The results indicated that the crude enzyme fraction is having effective amylolytic and lipolytic activities. These enzymatic activitieis of the extract substantiate the validity of claims made in traditional system of medicine about this plant fruits as a good appetizer bitter stomachic and carminative.

Momordica charantia[1] is a tender stemmed tendril climber of the cucurbitaceae family. This plant is found in the tropical regions throughout the world. The fruits of this plant are elongated and narrowed to both ends ribbed with prominent tubercles[2.] The fruit is commonly known as karela fruit and kaippakai (mal.) or pavakkai (Tam.) or hagalakkai (Kan) in South India.

In the traditional system of medicine the juice of the fruit, leaves, seeds and root are used for curing various ailments3. Chopra et al reported that the juice of the leaves are useful as an emetic and purgative, fruit juice as a stomachic and anthelminitic and root extract is useful in haemorrhoids[1].

Literature survey[3] revealed that karela fruits contain a lot of medicinally active compounds like diosgenin , gamma amino burytric acid, inulin, P&V insulin and various cytostatic factors. The reports[4] again indicated the presence of certain glycosides called as momordicosides H.I.J.K.L, various amino acids and phytosterols in these fruits.

Determination of Protein content [6,7]:

The protein content in the crude enzyme extract was determined by Folin-Lowry method[6]. Egg albumin (0.1-1.0 mg/ml) was used to prepare the standard curve, where absorbance is plotted against the concentration of protein at 540 nm. The absorbance of the crude enzyme extract was determined and extrapolated to obtain the amount of protein present in the extract.

Screening of Amylase activity[8]:

The amylase activity in terms of potency of the crude enzyme extract was determined according to a literature[9] method. The potency of crude the enzyme extract was calculated in nuits/mg. The results are tabulated in Table 1.

Qualitative Chemical Tests for Thiol groups, Disulphide and amide linkages[10,11]:

Qualitative chemical tests for the thiol groups, disulphide and amide linkages were performed according to the literature[10,11] to confirm the presence of enzymatic substances in the extract.

Estimation of Thiol Content[12]:

The thiol content in the amyloytic enzyme present in the crude enzyme extract was determined by iodometric titration. Thiols react with iodine to from disulphides. Iodine solution in excess was used as a oxidising agent in this reaction. The unreached iodine is determined by titration with standard thiosulphate solution. The percentage thiol was calculated and tabulated in Table 2.

Effect of temperature on the enzyme extract:

This experiment was conducted to find out the most suitable temperature at which the enzyme extract is showing maximum stability and potency. For this purpose a temperature range 25-40o was selected and at each temperature the potency of the crude enzyme was calculated. The results are tabulated in Table3.

Estimation of Lipolytic activity[13]:

The Lipolytic activity of the crude enzyme extract was screened according to the literature13. Clear vegetable oil (2ml) is neutralized to pH7 and stirred well with 25 ml of water in the presence of 100 mg bile salt and the total mixture is used as a substrate in this procedure. The enzyme activity is defined as the amount of enzyme which releases one milli-equivalent of free fatty acids min/g of sample. The results are tabulated in Table 4.

RESULTS AND DISCUSSION:

The protein content in the crude enzyme extract was found to be 0.98 mg/ml. The amylotic potency of the enzyme extract of Momordica charantia fruits was observed as 342.26 ± 21.04 u/g of the crude enzyme extract. The potency of the extract was found to be optimum at 35^{o}C and at pH of 7.8 for phosphate buffer. The lipolytic activity was expressed as meq/min/g of the sample. The crude enzyme extract showed a lipolytic activity of 0.01636 meq/min/g.

These observations throws light towards the efficiency of the crude enzyme extract obtained from the unripe fruits of *Momordica charantia Linn* as a good amyloltic and lipolytic agent . These activities of the enzyme extract can be utilized to make an effective digestive or carminative type of herbal formulations. Further studies are in progress to separated and purify various specific enzymes responsible for the amylolytic and lipolytic activities present in these fruits.

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TABLE 1: SCREENING OF AMYLASE ACTIVITY

A (ML)	B (ML)	A-B (ML)	Activity= $\frac{100}{[\{1/5(a-b)\}-0.006]w}$ units/mg	Mean ± S.D
17.0	17.1	0.1	357.14	
16.9	17.0	0.1	357.14	342.26 ±21.04
17.0	17.11	0.11	312.5	

A and B represent volume in ml of 0.1 M of sodium thiosulphate used in the titration against the test sample and the blank. W is 20 mg of the test sample used.

TABLE 2: ESTIMATION OF THIOL CONTENT PRESENT IN THE ENZYME EXTRACT

A (ML)	B (ML)	A-B (ML)	% thiol= $\frac{(A-B)xNxMx100}{X}$	Mean ± S.D
106.3	103.0	15.0	70.54	69.806 ± 2.743
106.2	103.2	15.0	66.14	
106.6	103.3	15.0	72.74	

A is the quantity of sodium thiosulphate consumed for blank. B is the quantity of sodium thiosulphate consumed for sample. X is the mass of the sample and M is the molecular mass of the thiol group (33.068).

TABLE 3: EFFECT OF TEMPERATURE OFN THE CRUDE ENZYME EXTRACT

Temp (oC)	A(ml)	B(ml)	A-B (ml)	Activity= $\frac{100}{[\{1/5(a-b))\}-0.006]w}$ units/mg
25	17.4	17.6	0.2	147.97
30	16.8	17.0	0.2	147.97
35	17.0	17.1	0.1	357.14
40	17.1	17.4	0.3	92.59

A and B represent volume in ml of 0.1 M of sodium thiosulphate used in the titration against the test sample and the clank. W is 20 mg of the test sample used.

TABLE 4: LYPOLYTIC ACTIVITY SCREENING STUDIES

Wt. of sample in g	Vol. of alkali consumed (ml)	Time in min	Enzyme activity= $\frac{\text{Vol. of alkali consumed x strength of alkali}}{\text{Wt. of sample in g x Time in min}}$	Mean ± S.D.
0.5	0.8	10	0.016	0.01636 ± 0.0007
0.5	0.82	9.45	0.0173	
0.5	0.8	10.10	0.0158	

W is 0.5 gm of the sample used
Strength of NaOH used in experiment is 0.1.N.