

This chapter deals with the evaluation of alpha amylase inhibitory activity of different extracts isolated from bulbs of *Allium cepa* L. , bulbs of *Allium sativum* L. and stem bark of *Mangifera indica* L. collected from Bharatpur and Jaipur districts of Rajasthan using different biochemical methods.

MATERIAL AND METHODS

Plant collection and authentication

Bulbs of *Allium cepa* Linn. , bulbs of *Allium sativum* L. and stem bark of *Mangifera indica* L. were collected from different localities of Jaipur and Bharatpur districts. Plant collection and authentication is same as discussed in chapter 1.

Extractions: Extraction of plant parts in different polar and non polar solvents (Water, Methanol, Ethanol, Acetone, Pet ether and Toluene) and for their secondary metabolites (Flavonoids and Alkaloids) was carried out as discussed in chapter 1.

Salivary alpha amylase assay

Starch – Iodine color assay and Glucose DNSA assay were used to estimate alpha amylase inhibitory activity of extracts. Procedures were followed as described in chapter 2.

Statistical Data Analysis

All experiments were performed for different sets each in triplicate. The data are expressed as mean \pm SEM (standard error of the mean). Statistical difference, Analysis of Variance (ANOVA) and linear regression analysis were performed using Graph pad prism 5 statistical software. The IC₅₀ values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by logarithmic regression analysis from the mean inhibitory values. The IC₅₀ values were defined as the concentration of the extract (containing the α -amylase inhibitor) that inhibited 50% of the alpha amylase activity.

Levels of percent inhibition of salivary alpha amylase enzyme by different extracts of *Allium cepa* L., *Allim sativum* L. and *Mangifera indica* L. are shown in Table 3.1.

Results:

***Allium cepa* L.**

Results revealed that various extracts of bulbs of *Allium cepa* Linn. exhibit alpha amylase inhibitory activity of different level.

Percent inhibition of alpha amylase enzyme by different extracts of *Allium cepa* L. and their IC₅₀ values are shown in Table 3.2 and 3.3 respectively in plants of Jaipur and Bharatpur districts. Percent inhibition in plants of Jaipur and Bharatpur districts is graphically represented in Figure 3.1 and Figure 3.2.

Water extracts of bulbs of *Allium cepa* L. (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) showed 44.74±0.26 % to 47.23±0.11 % inhibition with an IC₅₀ value of 0.005 g/ml in plants of Jaipur district and 44.55±0.22 % to 46.22±0.20 % inhibition with IC₅₀ value of 0.01 g/ml in plants of Bharatpur district.

Percent inhibition by methanol extracts (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 48.95±0.18 % to 50.59±0.33 % with an IC₅₀ value of 0.00094 g/ml and 48.02±0.16 % to 50.35±0.17 % with an IC₅₀ value of 0.001 g/ml in plants of Jaipur and Bharatpur districts respectively.

Ethanol extracts (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) were found to exhibit 43.68 ± 0.17 % to 45.35 ± 0.19 % inhibition with an IC_{50} value of 0.016 g/ml in plants of Jaipur district and 43.16 ± 0.13 % to 45.15 ± 0.10 % inhibition with an IC_{50} value of 0.059 g/ml in plants of Bharatpur district.

Acetone extracts (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) showed 54.93 ± 0.18 % to 56.34 ± 0.20 % inhibition (IC_{50} value 0.000003 g/ml) in plants of Jaipur district and 54.83 ± 0.20 % to 57.08 ± 0.15 % inhibition (IC_{50} value 0.000009 g/ml) in plants of Bharatpur district.

Percent inhibition by Pet ether extract (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) was 19.85 ± 0.27 % to 21.91 ± 0.24 % with an IC_{50} value 1137627.28 g/ml (In plants of Jaipur district) and 19.56 ± 0.13 % to 21.33 ± 0.21 % with IC_{50} value of 15881661891248.60 g/ml (In plants of Bharatpur district). Very high value of IC_{50} showed insignificant inhibition of salivary alpha amylase by the extract of plants collected from both districts.

Toluene extracts (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) were found to exhibit 11.67 ± 0.23 % to 13.40 ± 0.18 % inhibition (IC_{50} value 1137627.28 g/ml) in plants of Jaipur district and 11.25 ± 0.16 % to 13.24 ± 0.20 % inhibition (IC_{50} value 91201.08 g/ml) in plants of Bharatpur

district. Very high value of IC_{50} showed insignificant inhibition of salivary alpha amylase by the extract of plants collected from both districts.

Percent inhibition by Alkaloid extract (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 10.22 ± 0.06 % to 20.12 ± 0.10 % and 9.05 ± 0.14 % to 19.68 ± 0.07 % in plants of Jaipur and Bharatpur districts respectively. IC_{50} values of extracts were found to be 0.085 g/ml in plants of Jaipur district and 0.038 g/ml in plants of Bharatpur district.

Flavonoid extracts (at concentrations ranging from 0.3 mg/ml to 1.5 mg/ml) showed 56.22 ± 0.20 % to 58.33 ± 0.20 % inhibition (IC_{50} value 0.000022 g/ml) in plants of Jaipur district and 55.87 ± 0.06 % to 57.96 ± 0.23 % inhibition (IC_{50} value 0.000015 g/ml) in plants of Bharatpur district.

All experiments were performed in triplicates. One way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments p value was found to be lower than 0.05 which indicate that differences were significant. Percent inhibition and IC_{50} values were significantly different in different extracts but there was no significant difference in % inhibition and IC_{50} values of same extract of the plant at same concentration in two districts of Rajasthan.

***Allium sativum* L.**

Results revealed that various extracts of bulbs of *Allium sativum* Linn. exhibit alpha amylase inhibitory activity of different level.

Percent inhibition of alpha amylase enzyme by different extracts of *Allium sativum* L. and their IC_{50} values are shown in Table 3.4 and 3.5 respectively in plants of Jaipur and Bharatpur districts. Percent inhibition is graphically represented in plants of Jaipur and Bharatpur district in Figure 3.3 and Figure 3.4 respectively.

Water extracts showed (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) 25.19±0.16 % to 27.85±0.09 % and 27.12±0.15 % to 29.02±0.13 % inhibition of salivary alpha amylase in plants of Jaipur and Bharatpur districts respectively. IC_{50} value of the extract was 107.89 g/ml in plants of Jaipur district and 7.55 g/ml in plants of Bharatpur district. Very high value of IC_{50} showed insignificant inhibition of salivary alpha amylase by the extract of plants collected from Jaipur district.

Percent inhibition of alpha amylase by methanol extract (at concentration ranging from 0.3mg/ml to 1.5 mg/ml) was found to be 35.65±0.16 % to 37.54±0.12% with an IC_{50} Value of 0.959 g/ml (in plants of

Jaipur district) and $35.84 \pm 0.13\%$ to $38.14 \pm 0.17\%$ with an IC_{50} value of 27.54 g/ml (In plants of Bharatpur district).

Ethanol extracts of plant (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed $37.15 \pm 0.19\%$ to $39.07 \pm 0.11\%$ inhibition (IC_{50} value 9.09 g/ml) in plants of Jaipur district and $37.55 \pm 0.18\%$ to $40.08 \pm 0.19\%$ inhibition (IC_{50} value 1109.17 g/ml) in plants of Bharatpur district. Very high value of IC_{50} showed insignificant inhibition of salivary alpha amylase by the extract of plants collected from Bharatpur district.

Acetone extracts showed $44.13 \pm 0.12\%$ to $46.26 \pm 0.17\%$ and $45.14 \pm 0.17\%$ to $47.56 \pm 0.10\%$ inhibition of salivary alpha amylase (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) in plants of Jaipur and Bharatpur districts respectively. IC_{50} value of extract is 0.033 g/ml in plants of Jaipur district and 0.005 g/ml in plants of Bharatpur district.

Percent inhibition of alpha amylase by pet ether extracts (at concentration of 0.3 mg/ml to 1.5 mg/ml) was found to be $28.17 \pm 0.17\%$ to $30.57 \pm 0.11\%$ with an IC_{50} value of 95.72 g/ml (in plants of Jaipur district) and $28.97 \pm 0.16\%$ to $31.66 \pm 0.18\%$ with an IC_{50} value of 100.00 g/ml (in plants of Bharatpur district).

Toluene extracts of plant (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed 32.16 ± 0.14 % to 34.04 ± 0.13 % inhibition (IC_{50} value 7.55 g/ml) in plants of Jaipur district and 32.85 ± 0.14 % to 35.12 ± 0.18 % inhibition (IC_{50} value 75.85 g/ml) in plants of Bharatpur district.

Alkaloid extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) were found to exhibit 10.58 ± 0.16 % to 16.44 ± 0.16 % inhibition of alpha amylase with IC_{50} value of 1.58 g/ml in plants of Jaipur district and 10.14 ± 0.11 % to 16.37 ± 0.10 % inhibition of the enzyme with an IC_{50} value of 0.34 g/ml in plants of Bharatpur district.

Percent inhibition of alpha amylase by flavonoid extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 44.04 ± 0.18 % to 46.35 ± 0.14 % with an IC_{50} value of 0.014 g/ml in plants of Jaipur district and 45.12 ± 0.16 % to 47.83 ± 0.11 % inhibition with an IC_{50} value of 0.005 g/ml in plants of Bharatpur district.

All experiments were performed in triplicate. One way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments p value was found to be lower than 0.05 which show that differences were significant. Percent inhibition and IC_{50} values were significantly different in different extracts but there was no significant

difference in % inhibition and IC₅₀ values of same extracts of the plant at same concentration in two districts of Rajasthan. However, IC₅₀ values of water, ethanol and alkaloid extracts of plants showed significant difference in two districts of Rajasthan.

***Mangifera indica* L.**

Results revealed that various extracts of stem bark of *Mangifera indica* Linn. exhibit alpha amylase inhibitory activity of different level.

Percent inhibition of alpha amylase enzyme by different extracts of stem bark of *Mangifera indica* L. and their IC₅₀ values are shown in Table 3.6 and 3.7 respectively in plants of Jaipur and Bharatpur districts. Percent inhibition by extracts of plants of Jaipur and Bharatpur districts are graphically represented by Figure 3.5 and Figure 3.6 respectively.

Water extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed 39.46±0.09 % to 41.09±0.11 % and 40.16±0.15 % to 42.32±0.34 % inhibition of salivary alpha amylase in plants of Jaipur and Bharatpur districts respectively. IC₅₀ values of extracts were 0.19 g/ml and 0.18 g/ml in plants of Jaipur and Bharatpur districts respectively.

Percent inhibition of alpha amylase by methanol extracts (at concentration ranging from 0.3mg/ml to 1.5 mg/ml) was found to be 43.71±0.13 % to 46.09±0.15% with IC₅₀ value of 0.001 g/ml (in plants of Jaipur district) and 43.91±0.19 % to 47.28±0.25 % with IC₅₀ value of 0.001 g/ml (In plants of Bharatpur district).

Ethanol extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed 33.03±0.13 % to 35.65±0.07 % inhibition (IC₅₀ value 7.94 g/ml) in plants of Jaipur district and 33.44±0.23 % to 36.88±0.27 % inhibition (IC₅₀ value 0.59 g/ml) in plants of Bharatpur district.

Acetone extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed 44.55±0.22 % to 46.22±0.20 % and 44.74±0.26 % to 47.23±0.11 % inhibition of salivary alpha amylase in plants of Jaipur and Bharatpur districts respectively. IC₅₀ values of extracts were 0.001 g/ml and 0.005 g/ml in plants of Jaipur and Bharatpur districts respectively.

Percent inhibition of alpha amylase by pet ether extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 20.04±0.26 % to 22.44±0.46 % with an IC₅₀ value of 1000.00 g/ml (in plants of Jaipur district) and 20.25±0.11 % to 22.92±0.17 % with an IC₅₀ value of 70.79 g/ml (in plants of Bharatpur district). Very high value of IC₅₀ showed

insignificant inhibition of salivary alpha amylase by the extract of plants collected from Jaipur district.

Toluene extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) showed 16.81 ± 0.26 % to 18.98 ± 0.24 % inhibition (IC_{50} value 19498445.99 g/ml) in plants of Jaipur district and 17.06 ± 0.13 % to 19.21 ± 0.10 % inhibition (IC_{50} value 1995.26 g/ml) in plants of Bharatpur district. Very high value of IC_{50} showed insignificant inhibition of salivary alpha amylase by the extract of plants collected from both districts.

Alkaloid extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) were found to exhibit 1.16 ± 0.12 % to 17.33 ± 0.13 % inhibition of alpha amylase with IC_{50} value of 0.004 g/ml in plants of Jaipur district and 1.29 ± 0.10 % to 17.86 ± 0.13 % inhibition of the enzyme with an IC_{50} value of 0.003 g/ml in plants of Bharatpur district.

Percent inhibition of alpha amylase by flavonoid extracts (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 54.83 ± 0.20 % to 57.08 ± 0.15 % with an IC_{50} value of 0.000009 g/ml in plants of Jaipur district and 55.15 ± 0.14 % to 58.24 ± 0.13 % inhibition with an IC_{50} value of 0.000021 g/ml in plants of Bharatpur district.

All experiments were performed in triplicate. One way analysis of variance (ANOVA) was used to show significance of difference with respect to control. In all experiments p value was found to be lower than 0.05 which show that differences were significant. Percent inhibition and IC_{50} values were significantly different in different extracts but there was no significant difference in % inhibition and IC_{50} values of same extracts of the plant at same concentration in two districts of Rajasthan. However, IC_{50} values of pet ether extracts of plants showed significant difference in two districts of Rajasthan.