This chapter deals with the biosynthesis of silver nanoparticles synthesized by using water extracts of selected plant parts and flavonoid extract of *Aloe vera*, their characterization and evaluation of their salivary alpha amylase inhibitory activity.

Methodology

Plant material and preparation of the extracts

Green leaves of *Aloe vera* L., Bulbs of *Allium cepa* L, bulbs of *Allium sativum* L., Leaves of *Azadiracta indica* A Juss. and stem bark of *Mangifera indica* L. were collected from Jaipur district and were used to make aqueous extract. Each plant part (5gm) was thoroughly washed in distilled water, cut into fine pieces and was boiled into 100 ml sterile distilled water for 30 minutes, cooled at room temperatue and was filtered through Whatmann No. 1 filter paper (pore size 11 μm).

Flavonoids of *Aloe vera* Linn.were extracted by standered method as described in chapter 1.

Synthesis of silver nanoparticles

Silver nanoparticles were prepared from water extracts of all selected plants and from flavonoid extract of leaves of *Aloe vera* L. by using standard protocol (A. Singh et al., 2010). Plant extracts prepared were used for bioreduction of salt of silver nitrate. Aqueous solution of Silver nitrate (5μm) was prepared and used for the synthesis of silver nanoparticles. Ten milliliter of each plant extract was added into 90 ml of aqueous solution (5 mM) of Silver nitrate for reduction into Ag+ ions and was kept at room temperature for 4 hours. The reaction mixture was then centrifuged at 10,000 rpm for 15 minutes and washed three times with distilled water. Silver nanoparticles were kept for drying for further analysis.

Scanning electron microscopic (SEM) analysis of silver nanoparticles

The prepared silver nanoparicles were characterized using high resolution SEM analysis. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Samples were coated with gold to make the surface conductive and thin film of the sample was prepared on a carbon coated grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

Scanning electron microscopic photographs were obtained and analyzed (Fig. 5.1 to 5.6)

Determination of salivary alpha amylase inhibitory activity

Synthesized silver nanoparticles were evaluated '*in vitro*' for salivary alpha amylase inhibition at different concentrations (0.3-1.5 mg/ml). Silver nanoparticles each of with different concentration (0.3mg, 0.6 mg, 0.9 mg, 1.2 mg and 1.5 mg) were then mixed in 1ml of distilled water. These were sonicated and were used for spectrophotometric assay.

Salivary alpha amylase assay

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

Statistical Data Analysis

All experiments were performed in three different sets each in triplicate. The data are expressed as mean ± SEM (standard error of the mean). Statistical difference, ANOVA and linear regression analysis were performed using Graph pad prism 5 statistical software. IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and calculated by

logarithmic regression analysis from the mean inhibitory values. IC_{50} values were defined as the concentration of the extract, containing the α-amylase inhibitor that inhibited 50% of the alpha amylase activity.

Results:

Scanning Electron Microscopic analysis showed that all the nanoparticles synthesized were of good quality. Size of all nanoparticles was approximately 100nm.

Size and shape of silver nanoparticles synthesized by using different plant extracts were different which are mentioned in Table 5.1 and SEM images of nanoparticles are given in Figure 5.1 -5.6.

Results revealed that various silver nanoparticles showed alpha amylase inhibitory activity of different level. Percent inhibition of salivary alpha amylase activity and IC_{50} values are shown in Table 5.2 and graphically represented in Figure 5.7.

Percent inhibition of salivary alpha amylase enzyme by silver nanoparticles synthesized by using extracts of leaves of *Aloe vera* Linn. (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) was found to be 46.12±0.12 % to 50.57±0.10% with IC_{50} value of 0.00081gm/ml.

Silver nanoparticles synthesized by using extract of bulbs of *Allium cepa* Linn. (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) exhibited 42.12±0.10% to 46.74±0.11 % inhibition with IC_{50} value of 0.00067 gm/ml.

Silver nanoparticles synthesized by using extracts of bulbs of *Allium sativum* Linn. (at concentration ranging from 0.3 mg/ml to 1.5 mg/ml) were found to exhibit 44.43±0.11 to 48.63±0.11 % inhibition with IC_{50} value of 0.00060 gm/ml.

Percent inhibition by silver nanoparticles synthesized by using extract of leaves of *Azadiracta indica* A Juss. was found to be 45.18±0.12% to 49.23±0.10 % with IC_{50} value of 0.0023 gm/ml.

Silver nanoparticles synthesized by using extract of stembark of *Mangifera indica* Linn. were found to exhibit 44.17±0.09% to 48.65±0.11 % inhibition with IC_{50} value of 0.0026 mg/ml.

Percent inhibition by silver nanoparticles synthesized by using flavonoid extract of leaves of *Aloe vera* was found to be 45.13±0.11% to 50.16±0.10% inhibition with IC_{50} value of 0.0014 gm/ml.

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 ρ value was found to be lower than 0.05 which show that differences were significant. Percent inhibition and IC_{50} values are significantly different in different extracts.