Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia or increased blood glucose level with disturbance in carbohydrate metabolism resulting absolute or relative lack of insulin secretion (report of a WHO consultation, Geneva, 1999). The frequency of this disorder is on the rise globally and is likely to hit 300 million by 2025. India projected to have the largest number of diabetic cases (Gupta O.P. and Phatak S., 2003).

There are three forms of diabetes- Type I (Insulin dependent), Type II (Insulin independent) and Gastational. Type I diabetes is caused due to insulin insufficiency due to lack of functional beta cells (Cooke and Plotnick, 2008). Patients suffering from Type I are therefore totally dependent on exogenous source of insulin while patients suffering from Type II diabetes (insulin independent) are unable to respond to insulin and can be treated with dietary changes, exercise and medications (Elley and Kenealy, 2008). The most prevalent form both in the global and Indian scenario is the non insulin dependent diabetes mellitus which is associated with elevated postprandial hyperglycemia (WHO, 2006). Gestational diabetes is caused during pregnancy

The prevalence of Type II diabetes is on increase and it can be said that India is facing a diabetic explosion. Type II diabetes is characterized by resistance to the action of insulin and disorder of insulin secretion, either of

which may be the predominant feature (Alberti KG and Zimmet P.Z., 1998). Individuals with this type of diabetes do not need insulin to survive. Type II diabetes, which is the most common type, is often a result of excess body weight and physical inactivity in genetically predisposed individuals (Poulsen P *et al.*, 1999). Over time, diabetes can increase the risk of health-related problems including blindness, kidney damage, nerve damage, amputation of lower limbs and cardio vascular disease (De Coster VA, 2001). Although diabetes cannot be cured, the disease can be managed by non-pharmacological and pharmacological strategies, where improvements in glycaemic control are important factors in delaying the onset and progression of diabetes-related complications (DCCT research group, 1993; UKPDS Group, 1998).

The most prevalent form of diabetes affecting 90-95% of diabetics worldwide is Type II diabetes which is associated with elevated postprandial hyperglycemia (PPHG). During the last twenty years the prevalence of diabetes has increased dramatically in many parts of the world and the disease is now a worldwide public health problem. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild S *et al.*, 2004).

The treatment for this non-insulin dependent diabetes is presently achieved with the help of five classes of conventional drugs which act mainly by stimulation of insulin absorption and its release from pancreas or by the inhibition of carbohydrate degrading enzymes such as α-amylase and α-glucosidase (Rang *et al*., 2003). Most of these conventional drugs have varied side-effects because of which a search for natural enzyme inhibitors and their scientific evaluation from plant sources is increasing rapidly.

Alpha amylase (E.C.3.2.1.1) belongs to the class of α-1,4-glucan-4-glucanohydrolases is one of the important target enzymes for the conventional treatment of diabetes. There are two types of alpha amylase in body of human beings- salivary alpha amylase and pancreatic alpha amylase. It catalyses the initial step in hydrolysis of starch to maltose and maltotriose which are then acted upon by α-glucosidases, broken down into glucose that gets absorbed by the brush border epithelium of the intestine and enters the blood stream. The condition that arises due to this excessive breakdown of starch by alpha amylase and alpha glucosidases is referred to as PPHG. The strategy employed by most of the conventional anti-diabetic drugs available in the market (acarbose, voglibose and miglitol) is by the inhibition of α-amylase and α-glucosidase enzymes (Gholamhosenian *et al*., 2008). These α-glucosidase inhibitors have gastrointestinal side effects such as bloating, abdominal

discomfort, diarrhea and flatulence (Cheng *et al*., 2005). Thus extensive search for naturally available amylase and glucosidase inhibitors is a promising field of present day research. Natural α-amylase and α-glucosidase inhibitors from traditionally valued medicinal and food plants can provide benefit by controlling PPHG without any side effects, posed by most of the conventional drugs available for diabetes (Farias *et al*., 2008).

Several antidiabetic drugs, such as acarbose, miglitol, voglibose, sitagliptin, nojirimycin and 1-deoxynojirimycin, target different glucosidases, viz; sucrase, maltase, alpha amylase and produce favourable effects on glycemic values after food intake (Kim Y *et al*., 2005). Although their safety and tolerability has been widely evaluated for common clinical use of these drugs but their lack of specificity has been seen to produce several gastrointestinal side effects like abdominal cramping, flatulence and diarrhea (Hsieh SH *et al*., 2011; Li C *et al*., 2011, Iwamoto Y *et al*., 2010 and Fujisawa T *et al*., 2005). Besides oral agents and insulin therapy, phytotherapy is an alternative tool that provides a range of natural resources with hypoglycemic effects. Large number of plants has been reported in literature which provides relief to people suffering with diabetes.

Natural alpha glycosidase and alpha amylase inhibitors are being investigated as new candidates to control hyperglycemia in diabetic patients. Different acute and sub chronic toxicity studies have been developed in animal models regarding consumption of Phase 2 with no side effects being reported (Harikumar KB *et al*., 2005 and Chokshi D, 2006) and safety studies in humans have also been carried out. For instance, the safety of "PhaseolaminTM 1600 diet", Phase 2 and Suco-Block® consumption was investigated and no significant side effects were found (Thom E, 2000; Udani J *et al*., 2007 and Koike T *et al*., 2005). To summarize, it could be stated that the principle advantage of carbohydrate digestive enzyme inhibitors of plant origin consists in not causing severe side effects and may also be beneficial in weight reduction in individuals consuming large amounts of starch (Vinson J, 2009 and Bedekar A *et al*., 2010).

Traditional systems of medicine continue to be widely practiced on many accounts i.e. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of side effects to currently used drugs for hyperglycemia that have increase emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.

Global estimates indicate that 80% population cannot afford the products of the Western Pharmaceutical Industries or their allopathic counter parts and have to rely upon the use of traditional medicines which are mainly derived from plant material. In many of the developing countries the use of the plant drugs is increasing because modern life saving drugs are beyond the reach of three quarters of the third world' s population although many such countries spend 40-50% of their total wealth on drugs and health care. As a part of the strategy to reduce the financial burden on developing countries, it is obvious that an increase use of plant drugs will be followed in the future.

Medicinal plants are essential part of human medicine, since the dawn of civilization and are the backbone of traditional medicine system in India (Nayak *et al*., 2011). They represent rich source of hypoglycemic agents. Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rate than the modern medicine (Mann *et al*., 2008). The 'World Health Organization' estimated that 80% of the population of developing countries still relies on traditional medicines, mostly plant based drugs for their primary health care needs (Ikegami *et al*., 2003; Izzo., 2004).

In recent years a number of studies have been carried out on secondary metabolites of plant origin. However, in most of the studies crude extracts from plants have been screened only at preliminary level against pancreatic alpha amylase without evaluation of inhibitory action of extracts on salivary alpha amylase which is an important step to establish any extract/metabolite as hypoglycemic.

In this connection, the current work presents an effort to screen different polar and non polar extracts, flavonoids, alkaloids isolated from leaves of *Aloe vera* L. and *Azadiracta indica* A Juss., bulbs of *Allium cepa* L. and *Allium sativum* L. and stem bark of *Mangifera indica* L.

Preliminary detection of secondary metabolites was carried out using standard protocols. Thereafter were extracted out and were evaluated for their salivary alpha amylase inhibitory activity '*in vitro*'. Most active compound/compounds were identified by using TLC, PTLC, MP, IR and GCMS study.

The field of nanotechnology is one of the most active areas of research in modern material sciences. Nanopatricles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnosis, antimicrobials and therapeutics (M. Rai *et al.*, 2009; J.L.

Elechiguerra *et al*., 2005). Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanotechnology is a field that is burgeoning day by day, making an impact in all spheres of human life. New applications of nanoparticles and nanomaterials are emerging rapidly (W. Jshn, 1999; H.S. Naiwa, 2000; C.J. Murphy, 2008). Nano crystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics, antimicrobials and therapeutics (M. Rai and A Yadav, 2009; J.L. Elechiguerra *et al*., 2005), Catalysis (R.M. Crooks, 2001) and micro-electronics (D.I. Gittins *et al*., 2000).

Silver nanoparticles have been investigated to have an anti diabetic effect. In a recent investigation, silver nanoparticles have been found to show salivary alpha amylase inhibitory activity.

In the present investigation, silver nanoparticles were synthesized using aqueous extracts of different plant parts (leaves of *Aloe vera* L. and *Azadiracta indica* A Juss., bulbs of *Allium cepa* L. and *Allium sativum* L. and stem bark of *Mangifera indica* L.) and flavonoid extract of leaves of *Aloe vera* Linn., characterized by Scanning Electron Microscope (SEM) and then were screened for their salivary alpha amylase inhibitory activity '*in vitro*'.

All the selected plants are well known to have medicinal properties. These were collected from different localities of Jaipur and Bharatpur districts. The study also includes the effect of climatic conditions on the activity of salivary alpha amylase of different extracts of selected plants, collected from two districts of Rajasthan (Jaipur and Bharatpur).

The whole study has been complied in form of chapters which are as follows:

Chapter 1

Extraction of different parts of plants in solvents of varied polarity and for their secondary metabolites:

This chapter deals with preliminary detection and extraction of alkaloids, flavonoids of selected plants (leaves of *Aloe vera* L., leaves of *Azadiracta indica* A Juss., bulbs of *Allium cepa* L., bulbs of *Allium sativum* L.and stem bark of *Mangifera indica* L.). Extraction of different plants has also been done in different polar and non polar solvents using well established methods.

Preliminary detection of alkaloids and flavonoids in leaves of Aloe vera, leaves of *Azadiracta indica,* bulbs of *Allium cepa,* bulbs of *Allium sativum* and stem bark of *Mangifera indica* collected from Jaipur and Bharatpur districts of

Rajasthan was carried out using preliminary phytochemical methods (Brain and Turner, 1975; Evans, 1996; Sofowara, 1993; Harborne, 1973; Burchard, 1889; Salkowaki, 1872). Crude extracts in solvent of different polarity (viz; water, ethanol, acetone, methanol, toluene and pet ether) were also extracted from the selected plant parts. All extracts were quantified for each gram of dried plant material

Quantity of extracts in plants collected from Jaipur district

***Aloe vera* L.**

Crude extract in polar and non polar solvents was recorded to be maximum in water (102.25 mg/g.d.w.) followed by ethanol (98.80 mg/g.d.w.), acetone (97.04 mg/g.d.w.), ethanol (71.96 mg/g.d.w.), toluene (32.52 mg/g.d.w.) and pet ether (26.87 mg/g.d.w.).

Alkaloid content in the leaves of the plant was recorderd to be 17.01 mg/g.d.w. whereas flavonoid content in leaves was recorderd to be 8.8 mg/g.d.w..

***Allium cepa* L.**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (83.28 mg/g.d.w.) followed by ethanol

(72.56 mg/g.d.w.), acetone (71.23 mg/g.d.w.), ethanol (64.59 mg/g.d.w.), toluene (22.17 mg/g.d.w.) and pet ether(18.26 mg/g.d.w.).

Alkaloid content was recorderd to be 28.60 mg/g.d.w. whereas flavonoid content was recorderd to be 2.30 mg/g.d.w. in bulbs of plant.

***Allium sativum* L.**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (62.60 mg/g.d.w.) followed by ethanol (59.02 mg/g.d.w.), acetone (55.6 1 mg/g.d.w.), ethanol (29.56 mg/g.d.w.), toluene (14.54 mg/g.d.w.) and pet ether (12.13 mg/g.d.w.).

Alkaloid content was recorderd to be 34.40 mg/g.d.w. whereas flavonoid content was recorded to be 5.0 mg/g.d.w. in bulbs of plant.

***Azadiracta indica* A. Juss**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (106.56 mg/g.d.w.), followed by ethanol (92.38 mg/g.d.w.), acetone (88.45 mg/g.d.w.), methanol (65.07 mg/g.d.w.), toluene (36.72 mg/g.d.w.) and pet ether (28.13 mg/g.d.w.).

Alkaloid content was recorderd to be 14.20 mg/g.d.w. whereas flavonoid

content was recorded to be 6.60 mg/g.d.w. in leaves of plant.

***Mangifera indica* L.**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (98.48 mg/g.d.w.) followed by acetone (88.14 mg/g.d.w.), ethanol (86.35 mg/g.d.w.), methanol (82.39 mg/g.d.w.), toluene (34.58 mg/g.d.w.) and pet ether (26.17 mg/g.d.w.).

Alkaloid content was recorderd to be 6.6 mg/g.d.w. whereas Flavonoid content was recorded to be 7.2 mg/g.d.w. in stem bark of plant.

Quantity of extracts in plants of Bharatpur district

***Aloe vera* L.**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (98.13 mg/g.d.w.) followed by ethanol (96.76 mg/g.d.w.), acetone (95.25 mg/g.d.w.), methanol (68.58 mg/g.d.w.), toluene (31.29 mg/g.d.w.) and pet ether (25.54 mg/g.d.w.).

Alkaloid content was recorded to be 16.53 mg/g.d.w. whereas flavonoid content was recorded to be 7.30 mg/g.d.w. in leaves of plant.

***Allium cepa* L.**

Crude extract in polar and non polar solvents was recorded maximum by using water as solvent (81.19 mg/g.d.w.) followed by ethanol (72.14 mg/g.d.w.), acetone (70.20 mg/g.d.w.), methanol (63.56 mg/g.d.w.), toluene (21.23 mg/g.d.w.) and pet ether (17.53 mg/g.d.w.).

Alkaloid content was recorded to be 26.53 mg/g.d.w. whereas flavonoid content was recorded to be 2.10 mg/g.d.w. in bulbs of plant.

***Allium sativum* L.**

Crude extract in polar and non polar solvents was recorded to be maximum by using water as solvent (61.83 mg/g.d.w.) followed by ethanol (59.42 mg/g.d.w.), acetone (56.71 mg/g.d.w.), methanol (27.43 mg/g.d.w.), toluene (13.16 mg/g.d.w.) and pet ether (11.29 mg/g.d.w.).

Alkaloid content was recorded to be 32.21 mg/g.d.w. whereas flavonoid content was recorded to be 4.65 mg/g.d.w. in bulbs of lant.

***Azadiracta indica* A Juss.**

Maximum content was recorded by using water as solvent (111.54 mg/g.d.w.), followed by ethanol (95.46 mg/g.d.w.), acetone (91.53 mg/g.d.w.), methanol (68.17 mg/g.d.w.), toluene (37.53 mg/g.d.w.) and pet ether (30.19 mg/g.d.w.).

Alkaloid content was recorded to be 15.93 mg/g.d.w. whereas flavonoid content was recorded to be 7.81 mg/g.d.w. in leaves of plant.

***Mangifera indica* L.**

Crude extract in polar and non polar solvents was recorded maximum by using water as solvent (108.58 mg/g.d.w.) followed by acetone (91.23 mg/g.d.w.), ethanol (89.43 mg/g.d.w.), methanol (86.28 mg/g.d.w.), toluene (37.17 mg/g.d.w.) and pet ether (29.54 mg/g.d.w.).

Alkaloid content was recorded to be 7.93 mg/g.d.w. whereas flavonoid content was recorded to be 8.74 mg/g.d.w. in stem bark of plant.

Analysis of variance (ANOVA) was used to show the significance of differences in amount of extracts on the basis of polarity and in different districts. Significant difference was observed in the amount of extracts in different polar and non polar solvents in same plant but the amount of same extract of same plant was not different significantly in two districts.

Chapter 2

Evaluation of alpha amylase inhibitory activity of different extracts of *Aloe vera* L. and *Azadiracta indica* A Juss. in two districts:

This chapter deals with the evaluation of alpha amylase inhibitory activity of different extracts isolated from leaves of *Aloe vera* L. and leaves of *Azadiracta indica* A Juss. collected from Bharatpur and Jaipur districts of Rajasthan using different biochemical methods.

Glucose-DNSA colour assay and starch Iodine colour assay were used to screen alpha amylase inhibitory activity. Percent inhibition and IC50 values were calculated for each extract by statistical analysis,.

All extracts were screened for their salivary alpha amylase inhibitory activity at concentration ranging from 0.3 mg/ml to 1.5 mg/ml.

***Aloe vera* L.**

Results reveal that various extracts of leaves of *Aloe vera* L. exhibit alpha amylase inhibitory activity of different level.

Water extracts showed 41.73±0.14% to 43.64±0.13% and 41.10±0.13% to 43.05±0.10 % inhibition of salivary alpha amylase activity from plants of Jaipur and Bharatpur districts respectively. IC_{50} values of the extracts were 0.57 g/ml and 0.063 g/ml in plants of Jaipur and Bharatpur district respectively.

Percent inhibition of alpha amylase by methanol extracts was found to be 43.77±0.14% to 45.66±0.14% with an IC_{50} Value of 0.091 g/ml (in plants of

Jaipur district) and 43.61±0.10% to 45.65±0.07% with an IC_{50} value of 0.091 g/ml (In plants of Bharatpur district).

Ethanol extracts showed 50.13±0.90 % to 52.38±0.17 % inhibition (IC_{50} value 0.00031 g/ml) in plants of Jaipur district and 50.12±0.10 % to 51.83±0.13 % inhibition (IC_{50} value 0.00032 g/ml) in plants of Bharatpur district.

Acetone extracts showed 39.94±0.11 % to 42.03±0.22 % and 39.12±0.10 % to 41.53±0.11 % inhibition of salivary alpha amylase in plants of Jaipur and Bharatpur districts respectively. IC_{50} values of extracts were 0.471 g/ml and 0.199 g/ml in plants of Jaipur and Bharatpur district respectively.

Percent inhibition of alpha amylase by pet ether extracts was found to be 16.12±0.14 % to 18.54±0.08 % with an IC_{50} value of 2290.86 g/ml (in plants of Jaipur district) and 16.12±0.18 % to 18.27±0.09 % with an IC_{50} value of 4879.77 g/ml (in plants of Bharatpur district). Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Toluene extracts showed 16.55±0.13 % to 20.10±0.16 % inhibition (IC_{50} value 4.74 g/ml) in plants of Jaipur district and 16.82±0.10 % to 20.01±0.12 % inhibition (IC_{50} value 807.23 g/ml) in plants of Bharatpur district. Very high

value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Alkaloid extracts were found to exhibit 7.36±0.10 % to 17.34±0.10 % inhibition of alpha amylase with IC_{50} value of 0.032 g/ml in plants of Jaipur district and 7.24±0.10 % to 17.20±0.12 % inhibition of the enzyme with an IC_{50} value of 0.043 g/ml in plants of Bharatpur district.

Percent inhibition of alpha amylase by flavonoid extracts was found to be 55.83±0.12 % to 57.70±0.09 % with an IC_{50} value of 0.00019 g/ml in plants of Jaipur district and 54.75±0.12 % to 57.11±0.15 % inhibition with an IC_{50} value of 0.000003 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 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 but there is no significant difference in % inhibition and IC_{50} values of extracts of same plant in two districts.

***Azadiracta indica* A Juss.**

Results reveal that various extracts of leaves of *Azadiracta indica* A Juss exhibit alpha amylase inhibitory activity of different level.

Water extracts of leaves of the plant exhibited 16.43±0.12 % to 19.47±0.20 % inhibition with an IC_{50} value of 21.37 g/ml in plants of Jaipur district and 16.63±0.10 % to 18.52±0.16 % inhibition with an IC_{50} value of 4786.30 g/ml in plants of Bharatpur district. Very high value of IC_{50} of extract of the plant showed insignificant inhibition of salivary alpha amylase in Bharatpur district.

Percent inhibition by methanol extracts was found to be 13.35±0.13 % to 15.16±0.17 % with an IC_{50} value of 426.57 g/ml and 12.53±0.13 % to 14.44±0.14 % with an IC_{50} value of 380.18 g/ml in plants of Jaipur and Bharatpur districts respectively. Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both district.

Ethanol extracts were found to exhibit 16.53±0.12 % to 18.53±0.14% inhibition with an IC_{50} value of 2290.86 g/ml in plants of Jaipur district and 15.95±0.17 % to 18.07±0.16 % inhibition with an IC_{50} value of 177827.91 g/ml in plants of Bharatpur district. Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Acetone extracts showed 15.12±0.11 % to 17.52±0.06 % inhibition (IC_{50} value 1174.89 g/ml) in plants of Jaipur district and 15.25±0.12 % to 17.12±0.23 % inhibition (IC_{50} value 1174.89 g/ml) in plants of Bharatpur district. Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Percent inhibition by Pet ether extracts was found to be 20.36±0.13 % to 24.57±0.19 % with an IC_{50} value 11.74 g/ml (In plants of Jaipur district) and 21.57±0.10 to 23.94±0.17 % with IC_{50} value of 295.12 g/ml (In plants of Bharatpur district). Very high value of IC_{50} of extract of the plants showed insignificant inhibition of salivary alpha amylase in Bharatpur district.

Toluene extracts were found to exhibit 16.35±0.07 % to 18.53±0.10 % inhibition (IC_{50} value 2290.86 g/ml) in plants of Jaipur district and 16.22±0.10 % to 18.55±0.18 % inhibition (IC_{50} value 2290.86 g/ml) in plants of Bharatpur district. Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Percent inhibition by Alkaloid extract was found to be 15.60±0.12 % to 19.69±0.0.09 % and 16.23±0.11 % to 18.99±0.12 % in plants of Jaipur and Bharatpur districts respectively. IC_{50} values were 16.66 g/ml and 2333.45 g/ml in plants of Jaipur and Bharatpur districts respectively. Very high value of IC_{50}

of extract of the plants showed insignificant inhibition of salivary alpha amylase in Bharatpur district.

Flavonoid extracts showed 42.74±0.1.03 % to 46.85±0.13 % inhibition (IC_{50} value 0.009 g/ml) in plants of Jaipur district and 45.11±0.25 % to 47.40±0.19 % inhibition (IC_{50} value 0.006 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 ρ 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 extracts of plants in two districts however, extracts in water and pet ether and alkaloids showed different level of percent inhibition in plants of two districts.

Chapter 3

Evaluation of alpha amylase inhibitory activity of different extracts of bulbs of *Allium cepa* L. & *Allium sativum* L. and stem bark of *Mangifera indica* Linn. in two districts of Rajasthan:

This chapter deals with the evaluation of percent alpha amylase inhibitory activity of different extracts of bulbs of *Allium cepa*, *Allium sativum* and stem bark of *Mangifera indica* collected from two districts of Rajasthan.

All extracts were screened for their salivary alpha amylase inhibitory activity at concentration ranging from 0.3 mg/ml to 1.5 mg/ml.

***Allium cepa* L.**

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

Water extracts of bulbs of *Allium cepa* L. showed 44.74±0.26 % to 47.23±0.11 % inhibition with an IC_{50} value of 0.00 5 g/ml in plants of Jaipur district and 44.55±0.22 % to 46.22±0.20 % inhibition with an IC_{50} value of 0.010 g/ml in plants of Bharatpur district.

Percent inhibition by methanol extracts was found to be 48.95±0.18 % to 50.59±0.33 % with an IC_{50} value of 0.00094 g/ml and 48.02±0.16 % to 50.35±0.17 % with an IC_{50} value of 0.001g/ml in plants of Jaipur and Bharatpur districts respectively.

Ethanol extracts 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 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 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 5 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} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Toluene extracts 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 91201.083 g/ml) in plants of Bharatpur district. Very high value of IC_{50} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Percent inhibition by Alkaloid extract 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 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 ρ value was found to be lower than 0.05 which indicate that differences were significant. Percent inhibition and IC_{50} values are significantly different in different extracts but there is no significant difference in % inhibition and IC_{50} values of extracts of plants in two districts.

***Allium sativum* L.**

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

Water extracts showed 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 is 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} of extract of the plants showed insignificant inhibition of salivary alpha amylase in Jaipur district.

Percent inhibition of alpha amylase by methanol extractwas found to be 35.65±0.16 % to 37.54±0.12% with an IC_{50} Value of 0.095 g/ml in plants of Jaipur district and 35.84±0.13% to 38.14±0.17% with an IC_{50} value of 27.54 g/ml in plants of Bharatpur district.

Ethanol extracts of plant showed 37.15±0.19 % to 39.07±0.11 % inhibition (IC_{50} value 9.099 g/ml) in plants of Jaipur district and 37.55±0.18 % to 40.08±0.19 % inhibition (IC_{50} value 1109.17 g/ml) in plants of Bharatpur district. Very high value of IC_{50} of extract of the plants showed insignificant inhibition of salivary alpha amylase in Bharatpur district.

Acetone extracts showed 44.13±0.12 % to 46.26±0.17 % and 45.14±0.17 % to 47.56±0.10 % inhibition of salivary alpha amylase in plants of Jaipur and Bharatpur districts respectively. IC_{50} value of extract is 0.033g/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 was found to be

28.17±0.17 % to 30.57±0.11 % with an IC_{50} value of 95.71 g/ml in plants of Jaipur district and 28.97±0.16 % to 31.66±0.18 % with an IC_{50} value of 100.00 g/ml in plants of Bharatpur district.

Toluene extracts of plant 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 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 0.346 g/ml in plants of Bharatpur district.

Percent inhibition of alpha amylase by flavonoid extracts 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 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 of same plant but there is no significant difference in % inhibition and IC_{50} values of same extracts of same plants in two districts however, extracts in water and ethanol showed different level of percent inhibition in plants of two districts.

***Mangifera indica* Linn.**

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

Water extracts 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. IC_{50} values of extracts were 0.199 g/ml and 0.181 g/ml in plants of Jaipur and Bharatpur districts respectively.

Percent inhibition of alpha amylase by methanol extracts was found to be 43.71±0.13 % to 46.09±0.15% with an IC_{50} Value of 0.010 g/ml in plants of Jaipur district and 43.91±0.19 % to 47.28±0.25 % with an IC_{50} value of 0.010 g/ml in plants of Bharatpur district.

Ethanol extracts showed 33.03±0.13 % to 35.65±0.07 % inhibition (IC_{50} value 7.943 g/ml) in plants of Jaipur district and 33.44±0.23 % to 36.88±0.27 % inhibition (IC_{50} value 0.594 g/ml) in plants of Bharatpur district.

Acetone extracts 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. IC_{50} values of extracts were 0.010 g/ml and 0.005 g/ml in plants of Jaipur and Bharatpur districts respectively.

Percent inhibition of alpha amylase by pet ether extracts was found to be 20.04±0.26 % to 22.44±0.46 % with an IC_{50} value of 1000.00 g/ml in plants of Jaipur district and 20.25±0.11 % to 22.92±0.17 % with an IC_{50} value of 70.79 g/ml in plants of Bharatpur district. Very high value of IC_{50} of extract of the plants showed insignificant inhibition of salivary alpha amylase in Jaipur district.

Toluene extracts 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} of extracts of the plants showed insignificant inhibition of salivary alpha amylase in both districts.

Alkaloid extracts 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 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 ρ 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 same plants in two districts however, pet ether extracts showed different level od percent inhibition in plants of two districts.

Chapter 4

Characterization and identification of the most active compound/ compounds from the selected plants:

This chapter deals with identification of the most active compound/ compounds from the most active extracts.

Flavonoid extracts of all the selected plant parts was observed to

have the best inhibitory activity '*in vitro*' against salivary alpha amylase enzyme. Hence, flavonoid extracts were selected for the identification of compound/compounds through TLC, PTLC, MP, IR spectrum and GC-MS studies.

TLC of flavonoids was carried out in solvent system Benzene: acetic acid: water (Be: A: W) in ratio of 125: 72: 3 and n-Butanol: acetic acid: water (B:A:W) in ratio of 4:1:5.

TLC of flavonoid extract of *Aloe vera* L. showed three spots of Rf values as 0.56, 0.80 and 0.93 in Be:A:W and 0.78, 0.64 and 0.83 in B:A:W.

TLC of flavonoid extract of *Allium cepa* L. showed two spots of Rf values as 0.56 and 0.93 in Be:A:W and 0.78 and 0.83 in B:A:W..

TLC of flavonoid extract of *Allium sativum* L. showed two spots of Rf values as 0.39, 0.56 and 0.80 in Be:A:W and 0.78 and 0.64 in B:A:W.

TLC of flavonoid extract of *Azadiracta indica* A Juss. showed two spots of Rf values as 0.64 and 0.80 in Be:A:W and 0.89 and 0.64 in B:A:W.

TLC of flavonoid extract of *Mangifera indica* L. showed two spots of Rf values as 0.64 and 0.93 in Be:A:W and 0.89 and 0.83 in B:A:W.

Spots of Rf values 0.58, 0.64, 0.80 and 0.93 in Be:A:W and 0.78, 0.89, 0.64 and 0.83 in B:A:W coincide with the corresponding flavonoids viz; Leuteolin, Apigenin, Quercetin and Kaempferol respectively.

Melting points of isolated samples of Rf values 0.58, 0.64, 0.80 and 0.93 were found similar to those of standard Leuteolin (330°C), Apigenin (340°C), Quercetin (309-311°C) and Kaempferol (271-273°C) respectively.

IR spectra of all the four identified flavonoids were found superimposed with authentic standard compounds (Leuteolin, Apigenin, Quercetin and Kaempferol) which further confirmed the presence of Leuteolin, Apigenin, Quercetin and Kaempferol in the extracts tested (Fig. 7.1-7.4).

Since flavonoid extract from stem bark of *Mangifera indica* L. showed maximum inhibitory activity on salivary alpha amylase and minimum IC50 value, it was subjected to GC-MS analysis to explore the chemical constituents. In all, 50 compounds were identified. The retention time, name, molecular weight and the structure of the components of the test extract were ascertained (Table 7.2, Fig. 7.4). GC-MS study confirm the presence of many phenolic compounds in the sample.

Chapter 5

Biosynthesis, characterization and screening of silver nanoparticles synthesized by using extracts of the selected plants:

This chapter deals with the biosynthesis of silver nanoparticles using aqueous extracts of selected plant parts (leaves of *Aloe vera* and *Azadiracta indica*, bulbs of *Allium cepa* and *Allium sativum* and stem bark of *Mangifera indica*), and flavonoid extract of *Aloe vera*, their characterization and evaluation of their salivary alpha amylase inhibitory activity.

Selected plant parts collected from Jaipur district were used to make the aqueous extract. Plant parts weighing 5g were thoroughly washed in distilled water, cut into fine pieces and were boiled into 100 ml sterile distilled water for 30 minutes, cooled at room temperatue and filtered through Whatmann No. 1 filter paper (pore size 11 µm). Flavonoids were also isolated from 5g leaves of *Aloe vera* Linn. by using slandered protocol as described in Chapter 1.

Silver nanoparticles were prepared by using standard protocol (A. Singh et al., 2010). Plant extracts prepared were used to bioreduction of salt of silver nitrate. 5µM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 10 ml of plant extracts was added into 90 ml of aqueous solution of 5 mM Silver nitrate for reduction into Ag+ ions

and 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 and further analysed.

The prepared silver nanoparicles were characterized using high resolution analysis. Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine.

By SEM analysis size of silver nanonoparticles were found to be ~100 nm. Shape of silver nanoparticles synthesized by using *Aloe vera* leaves, *Azadiracta indica* leaves and stem bark of *Mangifera indica* and flavonoid extract of *Aloe vera* were found to be spherical while hexagonal shape was observed of silver nanoparticles synthesized by using water extracts of bulbs of *Allium cepa* Linn. and *Allium sativum* Linn..

Synthesized silver nanoparticles were screend for their salivary alpha amylase inhibitory activity '*in vitro*'.

All extracts were screened for their salivary alpha amylase inhibitory activity at concentration ranging from 0.3 mg/ml to 1.5 mg/ml.

Results:

Percent inhibition of salivary alpha amylase enzyme by silver nanoparticles synthesized by using extracts of leaves of *Aloe vera* Linn. 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. 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. 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.

Nanoparticles synthesized by using extract of stem bark 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 of different types of silver nanoparticles were found to be different significantly.

Results of the present study indicate that one or the other out of 80 extracts tested showed some degree of activity against salivary alpha amylase enzyme, indicating hypoglycemic nature of the selected plants. Among 80 extracts, 11 extracts showed IC_{50} value less than 1 mg/ml, 11 extracts were observed to have IC_{50} value between 1 mg/ml to 15 mg/ml, 10 extracts showed IC_{50} value between 16 mg/ml to 100 mg/ml whereas 48 extracts showed IC_{50} value higher than 100 mg/ml.

Among all extracts, flavonoid extracts were found to have maximum inhibitory potential with the lowest IC_{50} values. Pet ether and Toluene extracts showed a very high IC_{50} values indicating their low inhibitory potential on salivary alpha amylase. However, Pet ether extract of *Azadiracta indica* leaves had a lower IC_{50} value than that of others.

In the present study, we compared the alpha amylase inhibitory activity in plants collected from two districts (Jaipur and Bharatpur) of Rajasthan.

Water extracts of *Azadiracta indica* leaves showed presence of maximum content in plants of both districts. In Jaipur district, Maximum amount of content of crude extracts were recorded in water (as solvents) in all plants but content decreased in other solvents with decrease in their polarity. Maximum amount of crude content was recorded in leaves of *Azadiracta indica* in water (106.56 mg/g.d.w.), followed by leaves of *Aloe vera* L. in water (102.25 mg/g.d.w.), stem bark of *Mangifera indica* in water (98.48 mg/g.d.w.) , *Allium cepa* in water (83.28 mg/g.d.w.) and bulbs of *Allium sativum* in water (62.60 mg/g.d.w.).

In Bharatpur district, maximum amount of crude content was recorded in leaves of *Azadiracta indica* in water (111.54 mg/g.d.w.), followed by stem bark of *Mangifera indica* in water (108.58 mg/g.d.w.), leaves of *Aloe vera* L. in water (98.13 mg/g.d.w.), *Allium cepa* in water (81.19 mg/g.d.w.) and bulbs of *Allium sativum* in water (61.83 mg/g.d.w.).

In Jaipur district, maximum percent inhibition was exhibited by flavonoid extract of stem bark of *Mangifera indica* (IC_{50} value 0.009 mg/ml) while in Bharatpur district flavonoid extracts of *Aloe vera* leaves showed maximum percent inhibition (IC50 value 0.003mg/ml).

One way analysis of variance (ANOVA) was used to show significance

of difference between results of Jaipur and Bharatpur district. P values were found to be higher than 0.05 which showed that there was no significant difference in both, the content and IC_{50} values of plants collected from two districts.

Present study endorsed that the selected plants are rich in having compounds with high anti diabetic potential. Hence the selected plants can be utilized in the formulation of drugs to prevent hyperglycemia. Further through nano particles the activity of compounds can be increased.