Diabetes is a metabolic disease which is diagnosed on the basis of sustained high concentration of glucose in the blood. According to the 'World Health Organization' (WHO) the current diagnostic criteria for diabetes are: 1) plasma glucose concentration measured after an overnight fast (above 7.0mmol/l) 2) plasma glucose concentration measured two hours after 75g oral glucose load (above 11.0mmol/l) as per the study of Genuth S *et al*., (2003), WHO (2006) and Report of Diabetes care (2003). Diabetes occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. There are three types of diabetes mellitus- Type I, Type II and Gastational diabetes mellitus.

Type I diabetes results from autoimmune mediated destruction of the beta cells of the pancreas. Insulin is vital for individuals with Type I diabetes to avoid keto acidosis, coma and death. Diabetes is classified clinically as Type I and is characterized by insulin deficiency.

Type II diabetes is characterized by resistance to the action of insulin and/or 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, is the most common type, occurs due to 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). 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 the 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.

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).

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 and 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 α-amylase and α-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). Hence extensive search for naturally available amylase and glucosidase inhibitors is a need of the day. Natural α-amylase and α-glucosidase inhibitors from traditionally valued medicinal and food plants can provide benefit by

controlling PPHG without 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, especially sucrase, maltase and 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 due to the common clinical use of these drugs, 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 source that provides a range of natural resources with hypoglycemic effects, a range of plants providing raw materials recommended for people with diabetes.

Natural alpha glycosidase and alpha amylase inhibitors are being investigated as new candidates to control hyperglycemia in diabetic patients, but few data are available regarding the negative effects they might produce. Thus, 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). In summary, 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).

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. Herbs are supposed to be safe but many unsafe and fatal side effects have recently been reported (Ikegami *et al*., 2003; Izzo., 2004).

Most popular medicinal species with hypoglycemic effect, have been mentioned such as *Allium cepa, Allium sativum, Aloe vera, Arctium lappa,Azadiracta indica, brassica sp., Centaurium umbellatum, Cynara cardunculus subsp. Scolymus, Gentiana sp., Glycyrrhiza glabra, Mangifera indica, Morus sp., Phaseolus vulgaris, Rubus sp., Salvia officinalis, Taraxacum officinale, Trigonella foenum-graecum, Vaccinium myrtillus.* Medicinal plants in different oral formulations were recommended to the diabetic patient, but the mechanisms for hypoglycemic activity still remained incompletely understood. Hence, there is an urgent need to study the hypoglycemic properties of herbs in a scientific way which will definitely be helpful in the treatment of Diabetes Mellitus.

A lot work has been done by researchers on various medicinal plants for assessing their hypoglycemic activities. *Basella rubra, Oxalis cormiculat* and *Cocculus hirsutus*, the three traditionally known wild food plants have been examined for porcine pancreatic amylase inhibitory potential (Jyothi *et al*., 2011). Seventeen Indian Ayurvedic medicinal plants have been screened for potent α-amylase inhibitory activity (Zinjarde *et al*., 2011).

The leaves of *T. populnea* were studied for the presence of amylase inhibitors (Sangeetha and Vedasree, 2011). Determination of antioxidant

capacity and α-amylase inhibitory activity of the essential oils from *citronella* grass and *lemongrass* have also been done (Jumepaeng *et al*., 2013). Betulinic acid and 3,5,7,4'-tetrahydroxy flavonone have been identified from seeds of *S. cumini*, that also showed strong inhibition against porcine pancreatic α-amylase (Karthic *et al*., 2008). Various crude extracts of *V. negundo* and *T. chebula* were also studied by alpha amylase inhibition assay (Devnani *et al*., 2013). Ethanolic extract and andrographolide of *A. paniculata* have also been studied for alpha amylase and alpha glycosidase inhibitory activity, followed by a confirmatory '*In vivo'* study on rats (Subramanian *et al*., 2008).

Review of literature indicates that crude extracts from different parts of selected plants have earlier been studied for their hypoglycemic activity but still meager work has been carried out as far as the hypoglycemic activity of specific metabolite (alkaloids and/or flavonoids) is concerned. Most of the research has been restricted on determination of inhibitory activity on pancreatic alpha amylase. Determination of pancreatic alpha amylase inhibitory activity has now become an inevitable step so as to explore them at industrial level for formulation of drugs which could replace the existing ones. Hence, most of the studies carried out so far could only reveal their hypoglycemic potential but are not helpful in establishing them as anti diabetic.

In the present study an effort has been made to screen different crude extracts in different polar and non polar solvents (water, methanol, ethanol, acetone, toluene and petroleum ether), alkaloids and flavonoids of the selected plants parts (leaves of *Aloe vera* Linn., bulbs of *Allium cepa* Linn., bulbs of *Allium sativum* Linn., leaves of *Azadiracta indica* A Juss. and stem bark of *Mangifera indica* Linn.) collected from two different districts of Rajasthan (Jaipur and Bharatpur) against salivary alpha amylase enzyme in terms of percent inhibition and IC_{50} value. For most of the extracts IC_{50} values recorded were very low, indicating therapeutic potential of the selected plants.

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, 43 extracts showed high inhibitory activity, 6 extracts were observed to have moderate potential of inhibitory activity, 8 extracts showed lower inhibitory activity whereas 23 extracts showed insignificant inhibitory potential with very high IC_{50} values

Preliminary detection of alkaloids and flavonoids were also carried out in different parts of the selected plants before extracting them for screening. Results reveal that all the selected plants showed the presence of these

metabolites.

Quantification of crude extracts, alkaloids and flavonoids from different parts of the plants reveals that content were in good amount in the plants selected. However amount varies in different plants (Table 1.1-1.5).

***Aloe vera* Linn.**

Aloe vera is in demand due to its medicinal properties. It has been used to treat various diseases like cancer, diabetes, fever and a variety of chronic and infectious diseases. The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from *Aloe vera* are widely used in the cosmetics and alternative medicine industries, being marketed as having rejuvenating, healing, or soothing properties. There is, however, little scientific evidence of the effectiveness or safety of *Aloe vera* extracts for either cosmetic or medicinal purposes, and what positive evidence is available is frequently contradicted by other studies (Ernst E,2000; Marshall JM, 1990, Boudreau MD *et al.*, 2006 and Vogler BK *et al.*,1999).

Reports regarding the '*in vivo*' anti diabetic effects of *Aloe vera* preparations are conflicting, with several studies demonstrating blood glucose lowering effects (kim k *et al.*, 2009) but other investigations achieving different outcomes depending on the plant species, part of the plant, mode of

preparation and the diabetic model used (Okyar *et al*., 2001). Nevertheless, the results obtained in two nonrandomized clinical trials (n=40 and n=76) showed an improvement in fasting blood glucose levels after 6 weeks of treatment with aloe gel juice (Yongchaiyudha S *et al*., 1996 and Bunyapraphatsara N. *et al*., 1996).

In an investigation, different crude extracts of *Aloe vera* were used to evaluate for their pancreatic alpha amylase inhibitory activity. Water extract of plant was found to exhibit 23.3% inhibition at a concentration of 2.5 mg/ml while methanol and acetone extracts were found to exhibit no inhibitory activity (Sudha P *et al*., 2011).

In a study, aqueous extract of leaves of *Aloe vera* was injected to alloxan treated mice, the results showed that the extract had the significant desired effect and was able to decrease the amylase activity significantly (Manish G *et al*., 2010).

Some clinical studies concluded that there is some preliminary evidence to suggest that oral administration of *Aloe vera* might be effective in reducing blood glucose in diabetic patients and in lowering blood lipid levels in hyperlipidaemia (Feily A *et al*., 2009). The plant has been reported to possess anti diabetic potential as it is known to lower blood glucose levels (Gupta R *et*

al., 2008; Tanko Y *et al*., 20008; Rizvi MMA *et al*., 2009 and Ishikawa *et al*., 2007).

In the present study crude extracts in different polar and non polar solvents from leaves of *Aloe vera* L. have been extracted and screened to evaluate their salivary alpha amylase inhibitory activity.

Results reveal maximum amount of content was recorded in water solvent (102 mg/g.d.w.) followed by ethanol, acetone, methanol, toluene and pet ether in plants collected from Jaipur district. Same trend was observed in plants collected from Bharatpur district.

Aloe vera leaves showed anti diabetic potential, as all 12 crude extracts in different polar and non polar solvents exhibited inhibitory activity of salivary alpha amylase (Table 2.1 and Table 2.2).

In all crude extracts of plant collected from Jaipur district, ethanol extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (50.13±0.90 to 52.38±0.17 %) with the lowest IC_{50} value (0.00031 g/ml) followed by Methanol (IC_{50} value 0.091 g/ml), Water (IC_{50} value 0.57 g/ml), Acetone extracts (IC_{50} value 0.741 g/ml), Toluene (IC_{50} value 4.74 g/ml) and Pet ether (IC_{50} value 2290.86 g/ml) extracts. Very high value of IC_{50} of toluene and pet ether extracts of the plants showed insignificant

inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different solvents was ethanol> methanol> water> acetone>Toluene> pet ether.

In all crude extracts of plant collected from Bharatpur district, ethanol extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (50.12±0.10 to 51.83±0.13 %) with the lowest IC_{50} value (0.00032 g/ml) followed by Water (IC_{50} value 0.063 g/ml), Methanol (IC_{50} value 0.091 g/ml) , Acetone extracts (IC_{50} value 0.19 g/ml), Toluene (IC_{50} value 807.23 g/ml) and Pet ether (IC_{50} value 4879.77 g/ml) extracts. Very high value of IC_{50} of toluene and pet ether extracts of the plants showed insignificant inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different solvents was ethanol> water> methanol> acetone>toluene> pet ether.

***Allium cepa* L.**

Allium species such as *Allium cepa* is used as foodstuff, condiment, flavoring and as folk medicine (F.M. EI- Demerdash *et al*.,2004). Onion is a popular folk remedy. It is rich in flavonoids and sulfur comounds that have perceived benefits to human health (Griffiths *et al.*, 2002). *Allium cepa* has been used in Ayurveda to treat various diseases due to its anti inflammatory,

anti cancer, anti oxidant, anti diabetic potential (Yang J *et al*., 2004). Due to presence of sulfur containing compounds mainly in the form of cysteine derivatives, viz; S-alkyl cysteine sulfoxide which are decomposed by the enzyme allinase into a variety of volatile compounds such as thioslfinates and polysulfides during extraction. Onion possess anti diabetic, antibiotic, hypocholesterolaemic, fibrinolytic and various other biological effects (Augusti, 1996).

The hypoglycemic activity of *Allium cepa* has been demonstrated in many clinical studies. The addition of raw onion to the diet for non-insulin-dependent diabetic subjects decreased the dose of anti diabetic medication required to control the disease (Bhushan S., 1984). Moreover, it was noted that oral administration of *Allium cepa* crude hydroalcoholic extract in animal models (alloxan-induced diabetic rats) produced a significant hypoglycemic activity and favorable good health effects which may be most probably attributed to improvement and/or regeneration of pancreatic beta-cells (Tej Eldin *et al*., 2009).

In a study, in alloxan-diabetic rats, diabetes was induced by increasing the level of plasma glucose by 199% of control level. After treating them with juice of onion , plasma glucose level was reduced by 70% (Yang J *et al*.,

2004).

Different extracts of *Allium cepa* were evaluated for their effect on pancreatic alpha amylase enzyme. Results revealed that ethanol extracts showed pancreatic alpha amylase inhibitory activity while aqueous, chloroform, hexane and pet ether extracts of *Allium cepa* had no inhibitory activity (B Dineshkumar, 2012). Ethanol extract of *Allium cepa* has been reported for pancreatic alpha amylase inhibitory effects with an IC_{50} value 16.36 mg/ml (Nickavar B *et al*., 2009).

Pancreatic alpha amylase inhibitory activity of ethanol extracts of six allium species (*A. Akaka, a. cepa, A. porrum, A. sativum, A. ampeloprasum and A. hirtifolium*) was compared and was observed that extract of *Allium cepa* (at concentration ranging from 11.8-36.0 mg/ml) was found to exhibit 25.96±0.25 to 80.94±0.34 % inhibitory activity with an IC_{50} value of 16.36 mg/ml (B Nickavar *et al*., 2009).

In the present study crude extracts of bulbs of *Allium cepa* in different polar and non polar solvents have been quantified and screened to evaluate their salivary alpha amylase inhibitory activity.

Maximum amount of extract was obtained in water (83.28 mg/g.d.w.) followed by ethanol, acetone, ethanol, toluene and pet ether in plants collected

from Jaipur district. Similar trend was observed in plants collected from Bharatpur district.

Allium cepa bulbs showed anti diabetic potential, as all 12 crude extracts in different polar and non polar solvents exhibited inhibitory activity of salivary alpha amylase (Table 3.1 and Table 3.2).

In all crude extracts of plants collected from Jaipur district, acetone extract (concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (54.93±0.18 to 56.34±0.20 %) with the lowest IC_{50} value (0.000003 g/ml) followed by Methanol (IC_{50} value 0.944 mg/ml), Water (IC_{50} value 0.0059 g/ml), ethanol extracts (IC_{50} value 0.016 g/ml), Toluene (IC_{50} value 1023.29 g/ml) and Pet ether (IC_{50} value 1137627.28 g/ml) extracts. Very high value of IC_{50} of toluene and pet ether extracts of the plants showed insignificant inhibition of salivary alpha amylase.

In all extracts of plants collected from Bharatpur district, acetone extract (concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (54.83±0.20 to 57.08±0.15 %) with the lowest IC_{50} value (0.009 mg/ml) followed by Methanol (IC_{50} value 1.18 mg/ml) , Water (IC_{50} value 10.96 mg/ml), ethanol extracts (IC_{50} value 59.70 mg/ml) Toluene (IC_{50} value 91201083.93 mg/ml) and Pet ether (IC_{50} value 15488166189124600 mg/ml)

extracts. Very high value of IC_{50} of toluene and pet ether extracts of the plants showed insignificant inhibition of salivary alpha amylase.

The order of salivary alpha amylase inhibitory activity of different polar solvents was acetone> methanol> water> ethanol>Toluene> pet ether in both districts.

***Allium sativum* Linn.**

Allium sativum has attracted attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health, warding off illnesses and providing more vigor. The biological responses of garlic have been largely attributed to (i) reduction of risk factors for cardiovascular diseases and cancer, (ii) stimulation of immune function, (iii) enhanced detoxification of foreign compound, (iv) hepatoprotection, (v) antimicrobial effect and (vi) antioxidant effect (Banerjee and Maulik,2002).

Allium sativum is considered to be effective and one of the most commonly studied plant in relation to diabetes and their complications (Sujatha S, 2012). It has been suggested that extracts of *Allium sativum* control the blood glucose in serum and alter the activities of liver hexokinase glucose-6-phosphatase and hemoglobin coenzyme-A reductase towards normal.

Administration of aqueous extract of *Allium sativum* in the concentration of 10 ml/kg/day to rabbits significantly decrease blood sugar (Bhoyar P. *et al*., 2012).

In a study, level of plasma glucose was increased by 199% in alloxan-diabetic rats which was reduced by juice of *Allium sativum* by 68% (F.M. EI-Demerdash *et al*., 2004).

In another study, different crude extracts of rhizomes of *Allium sativum* were evaluated for porcine pancreatic alpha amylase (PPA) inhibitory activity but were found to have no inhibitory potential (Sudha P. *et al*., 2011).

Different crude extracts of bulb of *Allium sativum* were screened for their pancreatic alpha amylase potential. It was observed that aqueous, hexane, cyclohexane extracts showed no inhibitory activity but ethanol extract (at concentration ranging from 10-100mg/ml) showed to exhibit inhibitory effect ranging from 8.84±0.86 to 39.77±0.30 % with IC_{50} value 120.24 mg/ml (B Dineshkumar, 2012).

Literature reveals that ethanol extracts of six Allium species were evaluated for their pancreatic alpha amylase inhibitory activity '*in vitro*'. Extract of bulb of *Allium sativum* had 10.27±0.48 to 54.96±0.40 % inhibitions with an IC_{50} value of 17.95 mg/ml (B Nickavar *et al*., 2009).

In the present study crude extracts of bulbs of *Allium sativum* in different polar and non polar solvents have been extracted and screened to evaluate their salivary alpha amylase inhibitory activity.

Results reveal that maximum amount of extract was recorded in water solvent (62.60 mg/g.d.w.) followed by ethanol, acetone, ethanol, toluene and pet ether in plants collected from Jaipur district. Similar trend was observed in plants collected from Bharatpur district.

Allium sativum bulbs showed anti diabetic potential, as all 12 crude extracts in different polar and non polar solvents exhibited inhibitory activity of salivary alpha amylase (Table 4.1 and Table 4.2).

In all crude extracts of plants collected from Jaipur district, acetone extract showed maximum percent inhibition (44.13±0.10 to 46.26±0.17 %) with the lowest IC_{50} value (33.65 mg/ml) followed by Methanol (IC_{50} value 0.959 g/ml), Toluene (IC_{50} value 7.55 g/ml), ethanol extracts (IC_{50} value 9.09 g/ml), Pet ether (IC_{50} value 95.71 g/ml) and Water (IC_{50} value 107.89 g/ml) and extracts. Very high value of IC_{50} of water extract of the plants showed insignificant inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different polar and non polar solvents was acetone> methanol >Toluene>ethanol> pet ether> water.

In all crude extracts of plants collected from Bharatpur district, acetone extract again showed maximum percent inhibition (45.14±0.17 to 47.56±0.10 %) with the lowest IC_{50} value (0.005 g/ml) followed by Methanol (IC_{50} value 27.54 g/ml), Water (IC_{50} value 31.88 g/ml), Toluene (IC_{50} value 75.85 g/ml), Pet ether (IC_{50} value 100.00 g/ml) and ethanol extracts (IC_{50} value 1109.17 g/ml). Very high value of IC_{50} of ethanol extract of the plants showed insignificant inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different polar solvents was acetone> methanol> water >Toluene> pet ether> ethanol.

***Azadiracta indica* A Juss**.

In India, the plant is variously known as 'sacred tree', 'heal all', 'natures drugstore', village pharmacy' and 'panacea for all disease'. Products made from neem trees have been used in India for over two millennia for their medicinal properties: neem products are believed to be anthelmintic, antifungal, antidiabetic, antiviral, contraceptive and sedative. It is considered a major component in Ayurvedic and Unani medicine and is particularly prescribed for skin diseases (S. Zillur Rahmam *et al.*, 1996).

Hyperglycemic effect is observed with *Azadiracta indica* when given as leaf extract and seed oil comparable to that of glibenclamide. A. indica could

be of benefit in diabetes mellitus for controlling the blood sugar or may also be helpful in preventing or delaying the onset of the disease (Bhoyar P. *et al*., 2012).

In a study, alloxan induced mice were treated with *A. indica* leaf extracts for four weeks. It was observed that percentage fall in blood glucose was 29% and 34% after 2 and 4 weeks (P. Khosla *et al*., 2000).

In an investigation, ethyl ether fraction of chloroform extract of leaf of *Azadiracta indica* when tested to screen inhibitory activity against porcine pancreatic alpha amylase, IC_{50} value was found to be 0.046 mg/ml (Rosa MPG *et al*., 2012).

B. Dineshkumar (2012) evaluated porcine pancreatic alpha amylase inhibitory effect of aqueous, ethanol, hexane, pet ether and chloroform extracts of *A. indica* leaves in vitro. Results revealed that ethanol extract (at a concentration of 10-100µg/ml) had maximum inhibitory effect ranging from 16.50±1.23% to 66.66±0.93% with an IC_{50} value of 0.063 mg/ml. Aqueous extract showed minimum inhibitory activity (14.89±0.75 to 40.53±0.65) with an IC_{50} value of 0.124 mg/ml while other extracts had no inhibitory activity on PPA.

In the present study crude extracts in different polar and non polar

solvents from leaves of *Azadiracta indica* A Juss have been extracted and screened to evaluate their salivary alpha amylase inhibitory activity in the two districts of Rajasthan.

Maximum amount of exudate was recorded in water solvent followed by ethanol, acetone, methanol, toluene and pet ether in plants collected from both Jaipur and Bharatpur districts (Table 1.4).

Azadiracta indica leaves showed anti diabetic potential, as all 12 crude extracts in different polar solvents exhibit inhibitory activity of salivary alpha amylase (Table 5.1 and Table 5.2).

Out of all polar and non polar solvents extracts of plants collected from Jaipur district, ethanol extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (16.53±0.12 to 18.53±0.14 %) with the lowest IC_{50} value (IC_{50} value 0.22 g/ml) followed by Pet ether (IC_{50} value 11.74 g/ml), Water (IC_{50} value 21.37 g/ml), Methanol (IC_{50} value 426.57 g/ml), acetone (IC_{50} value 1174.89 g/ml) and Toluene (IC_{50} value 2290.86 g/ml) extracts. Very high value of IC_{50} of methanol, acetone and toluene extracts of the plants showed insignificant inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different polar and non polar solvents was ethanol> pet ether> water> methanol>acetone> toluene.

Out of all polar and non polar solvents extracts of plants collected from Bharatpur district, pet ether extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (21.57±0.10 to 23.94±0.17 %) with the lowest IC_{50} value (295.12 g/ml) followed by Methanol (IC_{50} value 380.18 g/ml), acetone (IC_{50} value 1174.89 g/ml), Toluene (IC_{50} value 2290.86 g/ml), Water (IC_{50} value 4786.30 g/ml) and ethanol (IC_{50} value 1778279.41 g/ml) . Very high value of IC_{50} of all extracts of the plant showed insignificant inhibition of salivary alpha amylase. The order of salivary alpha amylase inhibitory activity of different solvents was pet ether> methanol> acetone> toluene>water> ethanol.

***Mangifera indica* Linn.**

Studies indicate, anti diabetic, anti-oxidant, anti-viral, cardio tonic, hypotensive, anti-inflammatory properties of *Mangifera indica* Linn.. Various effects like antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, anti microbial, hepatoprotective, gastroprotective have also been studied (K.A. Shah *et al*., 2010).

A 50% ethanol extract of the leaves of *Mangifera indica* showed

significant hypoglycemic effect at a dose of 250 mg/kg, both in normal and streptozotocin-induced diabetic animals. The stimulation of β-cells to release insulin was thought to be part of the mechanism of action (Sharma SR *et al*., 1997). Investigations were carried out to evaluate the effect of *M. indica* on glucose absorption using a rat intestinal preparation '*in situ*'. The ethanol extracts of stem-bark reduced glucose absorption gradually during the whole perfusion period in Type II rats (Amrita B *et al*., 2009).

In a previous study, cold water , hot water , methanol , isopropanol , acetone, methyl tertiary butyl ether and cyclohexane extracts of fruits and leaves of *Mangifera indica* were evaluated in vitro for their Porcine pancreatic alpha amylase inhibitory activity but all extracts were found to show no inhibition (Sudha P. *et al*., 2011).

Petroleum ether, hexane, chloroform, ethanol and aqueous extracts of stem bark of *M. indica* were screened for their pancreatic alpha amylase inhibitory activity in vitro by B. Dineshkumar (2012). Ethanol extract (at a concentration of 10-100µg/ml) showed maximum inhibitory activity ranging from 35.79±0.33 to 62.49±0.34 % with IC_{50} value of 0.037 mg/ml. hexane extract showed minimum inhibitory activity ranging from 8.63±1.26 % to 40.24±0.34 % with an IC_{50} value of 0.114 mg/ml. Other extracts were found to

have no inhibitory activity on PPA.

In the present study crude extracts in different polar and non polar solvents from stem bark of *Mangifera indica* have been extracted and screened to evaluate their salivary alpha amylase inhibitory activity.

Results reveal that maximum amount of exudate was in water (98.48 mg/g.d.w.) followed by ethanol, acetone, methanol, toluene and pet ether in plants collected from Jaipur district whereas maximum amount of exudate was recorded in water (108.58 mg/g.d.w.) followed by acetone, ethanol, methanol, toluene and pet ether in plant collected from Bharatpur district.

Stem bark of *Mangifera indica* showed anti diabetic potential, as all 12 crude extracts in different polar and non polar solvents exhibit inhibitory activity of salivary alpha amylase (Table 6.1 and Table 6.2).

In all crude extracts of plants collected from Jaipur district, acetone extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (44.55±0.22 to 46.22±0.20 %) with the lowest IC_{50} value (10.96 mg/ml) followed by Methanol (IC_{50} value 10.96 mg/ml), Water (IC_{50} value 199.52 mg/ml), ethanol extracts (IC_{50} value 7943.28 mg/ml), Pet ether (IC_{50} value 10000000 mg/ml) and Toluene (IC_{50} value 949844599 mg/ml) . Very high value of IC_{50} of water, ethano, toluene and pet ether extracts of the

plants showed insignificant inhibition of salivary alpha amylase.

In all extracts of plants collected from Bharatpur district, acetone extract (a concentration ranging from 0.5 to 1.5 mg/ml) showed maximum percent inhibition (44.74±0.26 to 47.23±0.23 %) with the lowest IC_{50} value (5.94 mg/ml) followed by Methanol (IC_{50} value 10.96 mg/ml), Water (IC_{50} value 181.97 mg/ml), ethanol extracts (IC_{50} value 594.29 mg/ml) Pet ether (IC_{50} value 70794.57 mg/ml) and Toluene (IC_{50} value 1995262.314 mg/ml). Very high value of IC_{50} of toluene and pet ether extracts of the plants showed insignificant inhibition of salivary alpha amylase.

The order of salivary alpha amylase inhibitory activity of different solvents was acetone> methanol> water> ethanol > pet ether>Toluene which is similar in plants of both districts.

Medicinal use of alkaloid-containing plants has a long history. Many alkaloids are still used in medicine, usually in the form of salts, including antiarrhythmic, anticholinergic, cough medicine, remedy for gout, antipyretics, anti tumor *etc*.. Many synthetic and semisynthetic drugs are the modifications of alkaloids, which were designed to enhance or change the primary effect of the drug and to reduce unwanted side effects (Hesse *et al*., 2002).

An attempt has been made to evaluate alkaloids isolated from

Catharanthus roseus L. for their anti diabetic activity. All alkaloids induce relatively high glucose uptake in cells, implying their therapeutic potential against Type II diabetes (Soon H.T. *et al*., 2013.).

Hypoglycemic potential of alkaloids of selected plant parts have not been reported as yet. This is the first time when evaluated alkaloids of the selected plant parts have been studied for their salivary alpha amylase inhibitory activity '*in vitro'*.

In plants of Jaipur district, alkaloid content was found to be maximum in bulbs of *Allium sativum* (34.40 mg/g.d.wt.), followed by bulbs of *Allium cepa* (28.60 mg/g.d.wt.), leaves of *Aloe vera* (17.01 mg/g.d.wt.), leaves of *Azadiracta indica* (14.20 mg/g.d.wt.) and stem bark of *Mangifera indica* (6.60 mg/g.d.wt.).

Similar trend was observed in plants of Bharatpur district. Maximum content in bulbs of *Allium sativum* (32.21 mg/g.d.wt.), followed by bulbs of *Allium cepa* (26.53 mg/g.d.wt.), leaves of *Aloe vera* (16.53 mg/g.d.wt.), leaves of *Azadiracta indica* (15.93 mg/g.d.wt.) and stem bark of *Mangifera indica* (7.93 mg/g.d.wt.).

In plants collected from Jaipur district, maximum percent inhibiton was exhibited by alkaloids (a concentration ranging from 0.5 to 1.5 mg/ml) of

Mangifera indica ranging from 1.16+0.12 to 17.33+0.13 % with an IC50 value of 0.004 g/ml followed by *Aloe vera* (IC50 value 0.032 g/ml), *Allium cepa* (IC50 value 0.085 g/ml), *Allium sativum* (IC50 value 1.58 g/ml) and *Azadiracta indica* (IC50 value 16.66 g/ml).

In plants collected from Bharatpur district, maximum percent inhibiton was exhibited by alkaloids (a concentration ranging from 0.5 to 1.5 mg/ml) of *Mangifera indica* ranging from 1.29+0.10 to 17.86+0.13 % with an IC_{50} value of 0.003 g/ml followed by *Allium cepa* (IC_{50} value 0.038 g/ml), *Aloe vera* (IC_{50} value 0.043 g/ml), *Allium sativum* (IC_{50} value 0.34 g/ml) and *Azadiracta indica* (IC_{50} value 2333.45 g/ml). Very high value of IC_{50} of alkaloid extract of *Azadiracta indica* showed insignificant inhibition of salivary alpha amylase.

Flavonoids have been shown to have a wide range of biological and pharmaceutical activities in '*in vitro*' studies. These have been used as anti allergic, anti- inflammatory, anti-oxidant, anti microbial, anti cancer, anti diabetic , anti-diarrheal etc. (barjesteh S *et al*., 2007).

In a study, flavonoids were administered in alloxan treated mice, which increased the uptake of glucose by cells significantly (Ramulu jadhav *et al*., 2011).

A study was designed to study the salivary alpha amylase inhibitory

potential of flavonoid extracts of different parts of *Vitex negundo* Linn. and *Andrographis paniculata* Nees. where leaves of *Andrographis panniculata* showed maximum inhibitory activity with an IC_{50} value of 0.004 mg/ml (K Gautam., 2013).

Alpha amylase inhibitory activity of flavonoids of the selected plant parts have not been studied as yet. First time flavonoids of plants selected have been evaluated for their salivary alpha amylase inhibitory activity '*in vitro'*.

In Jaipur district, maximum flavonoid content was recorded in *Aloe vera* leaves (8.80mg/g.d.w.) followed by stem bark of *Mangifera indica* (7.20mg/g.d.w.), leaves of *Azadiracta indica* (6.60mg/g.d.w.), bulbs of *Allium sativum* (5.00mg/g.d.w.) and bulbs of *Allium cepa* (2.30 mg/g.d.w.).

In Bharatpur district, maximum flavonoid content was recorded in stem bark of *Mangifera indica* (8.74mg/g.d.w.) followed by leaves of *Azadiracta indica* (7.81mg/g.d.w.), *Aloe vera* leaves (7.30 mg/g.d.w.), bulbs of *Allium sativum* (4.65mg/g.d.w.) and bulbs of *Allium cepa* (2.10 mg/g.d.w.).

In plants collected from Jaipur district, maximum % inhibition of salivary alpha amylase by flavonoids (a concentration ranging from 0.5 to 1.5 mg/ml) was observed in stem bark of *Mangifera indica* (54.83±0.20 to 57.08±0.15 %) with an IC_{50} value of 0.000009 g/ml, followed by leaves of *Aloe*

vera (IC_{50} value 0.000019 g/ml), bulbs of *Allium cepa* (IC_{50} value 0.000022 g/ml), leaves of *Azadiracta indica* (IC_{50} value 0.009 g/ml) and bulbs of *Allium sativum* (IC_{50} value 0.014 g/ml).

In plants collected from Bharatpur district, maximum % inhibition of salivary alpha amylase by flavonoids (a concentration ranging from 0.5 to 1.5 mg/ml) was observed in leaves of *Aloe vera* (54.75±0.12 % to 57.11±0.15 %) with an IC_{50} value of 0.000003 g/ml, followed by bulbs of *Allium cepa* (IC_{50} value 0.000015 g/ml), stem bark of *Mangifera indica* (IC_{50} value 0.000021 g/ml), bulbs of *Allium sativum* (IC_{50} value 0.005 g/ml) and leaves of *Azadiracta indica* (IC_{50} value 0.006 g/ml).

One way analysis of variance was used to show the significance of difference among percent inhibition at different concentrations of different extracts. ρ values were found to be lower than 0.05 which showed that difference was significant.

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 as compared to other extracts.

In the present study, comparison has been made for 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 exudates in plants of both the districts, which decreased in other solvents with decrease in their polarity (water>ethanol> acetone>ethanol>toluene>pet ether).

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. (102.25 mg/g.d.w.), stem bark of *Mangifera indica* (98.48 mg/g.d.w.) ,bulbs of *Allium cepa* (83.28 mg/g.d.w.) and *Allium sativum* (62.60 mg/g.d.w.).

In plants of 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 (108.58 mg/g.d.w.), leaves of *Aloe vera* L. (98.13 mg/g.d.w.), bulbs of *Allium cepa* (81.19 mg/g.d.w.) and s*Allium sativum* (61.83 mg/g.d.w.).

In plants of Jaipur district, maximum percent inhibition was exhibited by flavonoid extract of stem bark of *Mangifera indica* (IC_{50} value 0.000009 g/ml) while in Bharatpur district flavonoid extracts of *Aloe vera* leaves showed

maximum percent inhibition (IC_{50} value 0.000003 g/ml).

One way analysis of variance (ANOVA) was used to show significance of difference between results of Jaipur and Bharatpur district. ρ values were found to be higher than 0.05 which showed that there was no significant difference between both, the content and IC_{50} values of plants collected from two districts.

In the present investigation, flavonoids were found to be the most active extracts, hence characterization of its compounds was carried out through TLC, PTLC, MP , IR spectrum and GC-MS studies.

TLC of bound flavonoids showed the presence of four spots of Rf values as 0.58, 0.64, 0.80, 0.93in solvent system Benzene: acetic acid : water (125:72:3) which were coinciding with the standard compounds leuteolin, apigenin, quercetin and kaempferol repectively. Melting points of isolated samples were found similar to those of satndered leuteolin, apigenin, quercetin and kaempferol (330°C, 340°C, 309-311°C and 271-273°C respectively).

IR spectra of all the four identified samples were found superimposed with authentic standard compounds (leuteolin, apigenin, quercetin and kaempferol) which further confirmed the presence ofleuteolin, apigenin, quercetin and kaempferol in the samples tested (Fig. 4.1-4.4).

Flavonoids of stem bark of *Mangifera indica* was analyzed through GC-MS study which showed the presence of many phenol compounds (Figure 4.5).

The study revealed that the selected plant parts are rich in active metabolites which are the precursor/source of anti diabetic drugs. Hence can be utilized in the formulation of synthetic and semisynthetic drugs to prevent hyperglycemia.

Nanopatricles 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). In a study, gold nanoparticles were synthesized by using aqueous extracts of *Cassia fistula* and were used in strptozotocin induced diabetic rats. The results of the study confirmed promising anti diabetic properties of gold nanoparticles (P Daisy *et al*., 2012).

In a recent investigation, silver nanoparticles were screened for their pancreatic alpha amylase inhibitory activity in vitro. Silver nanoparticles were synthesized using aqueous extract of *Sphaeranthus amaranthoides*. IC_{50} value was found to be 0.00028 mg/ml which showed hypoglycemic potential of silver nanoparticles (swarnalatha L. *et al*., 2012).

In the present study, silver nanoparticles have been synthesized through biological reduction of silver nitrate with aqueous extracts of the selected plant part (leaves of *Aloe vera*, leaves of *Azadiracta indica*, bulbs of *Allium cepa*, bulbs of *Allium sativum* and stem bark of *Mangifera indica*) and with flavonoids of *Aloe vera* Linn. collected from Jaipur district and were analysed through scanning electron microscope (SEM).

Biologically synthesized silver nanoparticles were then screened in vitro for their inhibitory potential on salivary alpha amylase enzyme. This was the first attempt to evaluate silver nanoparticles synthesized by the selected plant parts.

The morphology (shape and size) of different silver nanoparticles as per SEM analysis observation are mentioned in Table 5.1. Percent inhibitory potential and IC_{50} values are shown in Table 5.2 and Figure 5.7.

As per SEM analysis, size of all nanoparticles were found to be ~100 nm. Silver nanoparticles synthesized by using aqueous extracts of *Aloe vera* leaves, *Azadiracta indica* leaves, stem bark of *Mangifera indica* and flavonoid extract of *Aloe vera* were spherical in shape and were synthesized by using extract of bulbs of *Allium cepa* and *Allium sativum* which were found to be hexagonal in shape.

Silver nanoparticles exhibited inhibitory potential on salivary alpha amylase enzyme. Maximum percent inhibition was shown by silver nanoparticles synthesized by using bulbs of *Allium sativum* followed by bulbs of *Allium cepa,* leaves of *Aloe vera (aqueous),*leaves of *Aloe vera (flavonoids),* leaves of *Azadiracta indica and* stem bark *of Mangifera indica.*

Results of the study indicate the therapeutic potential of silver nanopartcles in the management of post-prandial hyperglycemia and Type II diabetes either alone or in a combinatorial therapy.