CHAPTER 6
SUMMARY

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the united states will have the largest number of people with diabetes. Diabetes mellitus is associated with impaired glucose metabolism that leads to an increase in free radical production and increase in triglycerides and lipoproteins levels. These free radicals may initiate lipid peroxidation which in turn stimulates protein glycation, inactivation of antioxidant enzymes and play an important role in diabetic complications. Hence among the therapeutic strategies, combination of antioxidant, anti hyperglycemic and wound healing activity can be beneficial in the prevention of diabetes mellitus and its associated complications.

*Chromolaena odorata* (L.) belongs to family Asteraceae is one of such plants that is being investigated for its diverse health benefits. *Chromolaena odorata* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, anti-microbial, cytoprotective and many other medicinally significant properties.

The proposed work was comprised of investigation of antioxidant, antidiabetic and wound healing activity of the *Chromolaena odorata* (L) King and Robinson since the plant was not explored for these activities so far.

Leaves of *Chromolaena odorata* were collected, powdered and obtained powder was subjected for standardization with different parameters. The standardized powders were subjected for the extraction with petroleum ether, chloroform, methanol and aqueous 40-60°C by soxhlet apparatus and with water by cold maceration method. These extract were concentrated, dried and the yield, color and consistency were recorded. These extracts were stored in a refrigerator at 2-8°C until usage.

Physico-chemical analysis were conducted in the selected plant sample recorded 10% of moisture, 11% of total ash, 24% of acid insoluble ash and 12% of
water soluble ash which determines the quality and purity of the plant drugs. Extractive value of methanolic extract of *C. odorata* was used in the present study as it provided high extractive value.

The various phytochemicals were detected in *C. odorata* leaf extracts. Alkaloids, flavonoids, phenolics, tannins, steroids, saponins, cardiac glycosides and carbohydrates were only present in methanolic extract and alkaloids, phenolics, tannins, steroids, saponins were present in aqueous extract whereas in petroleum ether and chloroform extract, alkaloids and flavonoids only present. Hence, the results revealed that methanolic extract was more efficient than other extracts.

The antioxidant activity of different extracts (Petroleum ether, chloroform, methanol and aqueous) of *Chromolaena odorata* leaves was investigated. Antioxidant activity was evaluated by using *in vitro* antioxidant assays like DPPH radical-scavenging activity, Superoxide radical-scavenging activity, Nitric oxide radical scavenging activity and Lipid peroxidation inhibiting assay. All the antioxidant activities were compared with standard antioxidant such as gallic acid. The methanolic extracts of the plant showed effective free radical scavenging activity with IC$_{50}$ values of 18.34, 50.14, 21.34 and 49.14, respectively.

The research has been carried out to evaluate potential of different extract of *Chromolaena odorata* leaf in inhibiting α-glucosidase and α-amylase activities. The methanolic leaf extract of *Chromolaena odorata* effectively inhibit these two enzymes. It was proposed that inhibition of the activity of such α-glucosidase and α-amylase would delay the degradation of carbohydrate, which would in turn cause a decrease in the absorption of glucose, as a result the reduction of postprandial blood glucose level elevation.

Thin layer chromatography and HPTLC techniques were used to separate individual chemical constituents of aqueous and methanolic extracts of *C. odorata*. The eluted compounds from methanolic extracts are rutin, catechin, caffeic acid and coumarin with Rf values of 0.20, 0.69, 0.90 and 0.74, respectively.

Methanolic extract of *Chromolaena odorata* was found safe (no mortality) at the dose of 1000 mg/kg and 2000 mg/kg body weight, hence 1/4th of this taken as lethal dose i.e. 250 mg/kg and 500 mg/kg.
Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration i.e., hyperglycemia, caused by insulin deficiency, often combined with insulin resistance. Hyperglycemia occurs because of uncontrolled hepatic glucose output and reduced uptake of glucose spills over into the urine (glycosuria) and causes an osmotic diuresis (polyuria), which in turn results in dehydration, thirst and increased drinking (polydypsia).

Overall results of present investigation show that methanolic extract of *Chromolaena odorata* possesses marked anti-hyperglycemic activity by improvement of glucose tolerance test and by lowering the blood glucose levels in streptozotocin-induced diabetic rats. These extracts had shown highly significant prevention of reduction in body weight.

The hyperglycemic effect comparable to glibenclamide suggested that the active fractions may act by regenerating the β cells in streptozotocin-induced diabetes. After administration of these extract to rats, it was found that TC, TG, LDL and VLDL level of all tested rats were significantly decreased and at the same time HDL level was increased. This provided evidence in favor of the view that these extracts could play an important role in treating diabetic/ hyperlipidemic patients.

The methanolic extract of *C. odorata*, when administered to the diabetic animals improved both SOD and CAT activities substantially, reflecting the antioxidant potency were found to be better than those of glibenclamide administered diabetic animals. Stabilization of serum creatinine and urea levels through administration of the extract *C. odorata* is further a clear indication of the improvement of the functional status of the liver cells. Histological examination of pancreas of these animals showed comparable regeneration of Islets of Langerhans and β cells by methanolic extract of *Chromolaena odorata* (250 and 500 mg/kg) and glibenclamide standard drug, which were earlier, necroses by streptozotocin.

The present study clearly demonstrates that the *C. odorata* extract enhances glucose uptake under *in vitro* conditions. This may be due to its effect on the number of receptors located in the skeletal muscle cell line and further studies with estimation of insulin and insulin receptors may give more insight into the mechanism of the antidiabetic activity of *C. odorata*. 
Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal stage and wound contraction is a process of shrinkage of area of the wound. It depends upon the reparative abilities of the tissue, type and extent of the damage and general state of health of the tissue. The granulation tissue of the wound is generally composed of fibroblasts, collagen, edema and small new blood vessels. The methanolic extract of Chromolaena odorata significantly speed up the healing process and provide the strength to collagen tissue. The preliminary phytochemical analysis of the leaf extract showed the presence of flavonoids, triterpenoids and alkaloids. Any one of the observed phytochemical constituents present in Leaf of Chromolaena odorata L may be responsible for the wound healing activity. Hence, the results of this study can be justified by the facts that the methanolic extract of leaf of Chromolaena odorata enhances the faster lay down of collagen fibres and improves the antioxidant status in the wound of diabetic animals.