The present research work comprises of the phytochemical and pharmacological investigations of leaves and testa (nut skin) of \textit{Anacardium occidentale} Linn. (cashew) for antidiabetic activity through \textit{in vitro} antioxidant assays and the \textit{in vivo} Streptozotocin induced type II diabetes model in rats.

The research work encompasses a detailed and systematic phytochemical and pharmacological investigation of various extract/s and fractions of testa and leaves of cashew. The plant was authenticated and a study of the microscopical and macroscopical characters and powder characteristics of the leaves were studied. Various physicochemical tests were performed to determine parameters like ash values, extractive values etc.

Extraction of the plant material was carried out by various techniques such as soxhlet extraction, decoction and a novel method for extraction with microwave assisted process (MAE) was developed and parameters were optimized. Ethanol, methanol and aqueous extracts of testa and aqueous, and ethanol extracts of leaves were prepared. MAE was found to increase the extractive yield from leaves about three times higher as compared to conventional techniques of extraction.

Qualitative phytochemical tests of the extracts of testa and leaves of cashew revealed the presence of carbohydrates, proteins, flavonoids, alkaloids, tannins and phenolic compounds. The extraction for leaves and testa were carried out with solvents of similar polarity. Thus, phytoconstituents of similar nature were found in ethanol, methanol and aqueous extracts of testa and leaves, except for saponin glycosides which were present in leaves and not in testa. Gums, mucilage, amino acids and inorganic compounds were not found to be present in both leaves and testa of cashew. Catechin was isolated from ethanol extract of testa by preparative thin layer chromatography. The purity and identity of the isolated catechin was confirmed by chromatographic analysis (HPTLC and HPLC) with reference standard of catechin.

Various chemical and spectral studies and chromatographic analyses were performed to ascertain the structure and identity of isolated catechin. $\%$ Purity of catechin was found to be 99.82\% by HPTLC and 99.65\% by HPLC.

A HPTLC and HPLC method was developed for chromatographic analysis of various extracts and quantitation of catechin in the prepared extracts and HPLC profiling and HPTLC fingerprinting was carried out.
The leaves of cashew were subjected to various drying conditions in order to study the effect of temperature on the polyphenol content and antioxidant activity of the extracts. Sun dried leaves were found to exhibit greater catechin content as compared to the shade dried, fresh and oven dried leaves extracts.

The extracts and fractions of cashew leaves and testa were subjected to various *in vitro* and cell line based antioxidant assays, in order to ascertain their antioxidant effect. *In vitro* assays viz. DPPH radical scavenging assay, Griess assay, anti-lipid peroxidation assay were carried out and ethanol extracts of testa and leaves of cashew exhibited better antioxidant activity as compared to other extracts.

In cell line based assays, the results of ROS assay for catechin, polyphenol fraction of testa and aqueous extract of testa, showed a concentration dependent decrease in production of ROS by oxidation of H$_2$DCFDA dye after 3 hrs incubation period, indicating a good antioxidant activity at cellular level in HMEC’s.

The cell proliferation reagent WST-1 was used in a colorimetric assay for the quantification of cell viability and proliferation. Pre-incubation with increasing concentrations of catechin, ethanol extract of cashew leaves, ethanol extract of cashew testa and polyphenols of cashew testa and leaves were found to rescue cell viability after H$_2$O$_2$ treatment to some extent, at selected concentrations.

As catechin exhibited significant activity in ROS assay and cell proliferation assay, it was selected for angiogenesis assay. Measurement of angiogenic capacity based on the mean tube length was observed for catechin after 24 hrs. Matrigel based assay indicated that catechin was not able to inhibit several key events of the angiogenic process thus exhibiting pro-angiogenic activity which is beneficial in treatment of diabetes related complications like atherosclerosis, peripheral arterial diseases, and wound healing disorders.

The expression of Nrf2 and beta-actin by proteins extracted from pretreated HMEC cells was measured by Western blot analysis. Expression of bands in the lane of standard Nrf2 lysate by proteins extracted from catechin treated cells were observed. This indicates the potential of catechin for Nrf2 activation which is a vital antioxidant response enzyme.

Expression of phase II enzymes is important in protecting the cells against stress conditions. Evaluation of mRNA expression profiles of phase 2 enzymes in catechin, tbHQ (positive control) and tbH$_2$O$_2$ treated cells using real-time PCR. Treatment of
human microvascular endothelial cells (HMECs) with 2.5 µM and 25µM concentration of catechin resulted in an upregulation of the Nrf2 target gene HMOX. Upregulation of the Nrf2 target gene HMOX was observed compared to tbHQ (positive control) and vehicle as the control. At a 25µM concentration of catechin for GCLC and NQO-1 a decrease was observed upto1.5 and 0.6 fold respectively.

Acute oral toxicity studies were carried out in albino mice following OECD 423 guidelines, for extracts which showed a better antioxidant activity in vitro. The crude extract/s and polyphenol fractions of leaves and testa of cashew did not produce toxic symptoms or changes in behavior or death. The extracts were found to be safe in mice upto the dose of 2000 mg/kg body weight, except for polyphenol fraction of leaves which was found to be safe upto 300 mg/kg dose.

Pharmacological investigation of antidiabetic activity of various extracts of cashew testa and leaves was carried out by STZ induced nicotinamide model in adult rats and STZ induced neonatal model.

In the evaluation of antidiabetic activity by STZ induced nicotinamide model in adult rats, ethanol extract of testa, polyphenols of cashew testa and ethanol extract of leaves, at single dose levels and ethanol extract of testa at double dose levels showed statistically significant results as compared with diabetic control in decreasing the fasting blood glucose levels. The lipid profile showed statistically significant results as compared with diabetic control for reduction in triglyceride, total cholesterol, and VLDL-C levels.

In STZ induced neonatal model, ethanol extract and polyphenols of cashew testa were evaluated for their antidiabetic effects, at single dose levels. The renal markers were accessed to ascertain the effect of drug treatment in diabetic rats. No significant difference was observed between the treatment group and diabetic control in levels of renal markers. The lowered lipid profiles levels for VLDL-C and LDL-C were found to be statistically significant as compared to diabetic control. However, a significant reduction in HDL-C was not observed. High levels of LDL cholesterol and low levels of HDL cholesterol (high LDL/HDL ratios) are risk factors for atherosclerosis, while low levels of LDL cholesterol and high levels of HDL cholesterol (low LDL/HDL ratios) are desirable and protect against heart disease and stroke. The administration of ethanol extract of testa and ethanol extract of leaves at dose levels of 175 mg/kg and 100 mg/kg did not decrease the HDL-C as compared to controls to a statistically
significant level, which is beneficial in the treatment of diabetes related complications like atherosclerosis. Thus, the results presented here, suggest that these extracts of cashew testa and leaves could be developed as a phytomedicine.

Catechin was found to be a potent bio-molecule in the cell line based assays and ethanol extract of testa is a polyphenol rich extract with potent antidiabetic activity. Development of an oral dosage form with catechin as the active drug would be expensive due to the involvement of isolation process. Hence, an economic and alternative oral tablet dosage form, from the bioactive ethanol extract of cashew testa was developed and evaluated.

The developed tablet dosage form was evaluated for various parameters like disintegration time, dissolution profile, content uniformity, friability and stability as per pharmacopoeial assays and ICH guidelines.

The developed tablets were found to comply with the stated pharmacopoeial limits for all the post compression parameters. The disintegration time for tablets was found to be less than 2.5 mins which is indicative of good release and water absorption properties of the drug. The cumulative % drug release was found to be 95.43% within one hour for the tablets during the dissolution testing which will be a beneficial factor for the tablet dosage form to be used as an antidiabetic agent.

In the stability studies of the formulation, the tablets were analyzed for 6 months period for drug content uniformity, hardness, in vitro disintegration time and friability. The tablets were found to be stable for 6 months period and they retained their original properties.

The developed tablet dosage form can serve as an important lead for herbal antidiabetic agents. The prepared tablets are economical as they are prepared from the extract of cashew testa which is a waste product of cashew processing industries. It is a safe, effective, economic and complimentary dosage form, to currently available synthetic antidiabetic drugs as it is herbal in origin and is found to be safe in the cell line based assays and animal experimentation carried out in the present research work.