2.1 BACKGROUND

Medicinal plants are the richest bioresources of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and leads for synthetic drugs. During the past 100 years, several hundred medicinal plants have been investigated with respect to plant chemistry, active chemical constituents, pharmacological effects, safety, and efficacy. In fact, these areas cover only a small fraction of the body of classical Ayurveda and complementary and alternative medicine (CAM) used worldwide. These areas offer little or no information about current biochemical, pharmacological, and clinical investigations. However, the basic research on Ayurvedic therapeutic agents has not been adequately integrated into disease management protocols. Scientific studies in laboratory animals have now confirmed the pharmacological properties of many medicinal plants. Experimentation should be initiated with the Ayurvedic or herbal medicines that hold a promise, at least for difficult-to-treat chronic diseases. Ayurveda and CAM’s cannot be ignored in the pursuit to make medicine evidence based. A new trans-disciplinary venture called Reverse Pharmacology has recently materialized and addresses both these needs. Reverse Pharmacology (RP), designed as a discipline to reduce three major issues of costs, time and toxicity. RP can be supposed to comprise of three phases. First, the experiential phase that include detailed documentation of clinical observations of the biodynamic effects of standardized Ayurvedic drugs through record keeping. Second, the investigative studies for tolerability, drug-interactions, dose-range finding in patients of defined subsets of the disease in relevant in vitro and in vivo models to evaluate the target-activity. Third phase includes experimental studies, basic and clinical, at several levels of biological organization, to identify and validate the reverse pharmacological correlates of Ayurvedic drug safety and efficacy. The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biology and to optimize safety, efficacy and acceptability of the leads in natural products. In this approach investigations travel a reverse path from ‘clinics to laboratory’ rather than classical ‘laboratory to clinics’. Although medicinal plants have been historically used for diabetes treatment throughout the world, few of
them have been validated by scientific criteria. Drug development for herbal drugs can follow different paths. Reverse pharmacology offers a major pattern of shift in drug discovery. The science has to incorporate documented clinical and experiential hits into leads by interdisciplinary exploratory studies on defined targets *in vitro* and *in vivo* and conducting an array of developmental activities (Patwardhan, 2008).

Recently, a large diversity of animal models has been developed to better understand the pathogenesis of diabetes mellitus and new drugs have been introduced in the market to treat this disease. In the majority of the studies, natural products mainly derived from plants have been tested in diabetes models induced by chemical agents. Preclinical experiments should be initially carried out *in vivo*, and be complemented, when possible, with *in vitro* studies to explore and advance in the mechanism of action of a natural product.

### 2.2 AIMS AND OBJECTIVES

The present research work encompasses the phytochemical and pharmacological investigations of leaves and testa (nut skin) of *Anacardium occidentale* Linn. (Cashew) for *in vitro* and *in vivo* antidiabetic activity through Streptozotocin induced type II diabetes model in rats.

To carry out phytochemical analyses and *in vitro* assays to estimate and identify bioactive extracts, fractions and some components of cashew.

The aims and objectives of the present research are as follows:

1. Screening of extract/s and fraction/s for antioxidant activity.
2. Phytochemical investigations of various extracts of cashew leaves and testa.
3. Acute oral safety studies of extract/s and fractions by OECD guidelines.
4. Evaluation of bioactive extract/s and fraction/s for antidiabetic activity and assessment of metabolic parameters like glucose and lipid profile.
5. Detailed evaluation of bioactive extract/s and or fraction/s on type 2 diabetes animal models.
6. Formulation of bioactive extract into suitable dosage form and their evaluation.
2.3 PLAN OF WORK

PART - I

1. **Literature survey:**
   To carry out relevant literature search regarding the plant *Anacardium occidentale Linn.* (Cashew).

2. **Procurement and Authentication of plant material:**
   To carry out authentication of plant material in order to ascertain its identity.

3. **Extraction of the plant material:**
   To prepare various extracts of cashew leaves and testa by conventional soxhlet extraction and microwave assisted extraction technique.

4. **Phytochemical investigation of the extracts:**
   To carry out qualitative phytochemical evaluation of the extracts for investigating the presence/absence of various phytoconstituents.

5. **TLC, HPTLC and HPLC studies of the extract/s and fraction/s:**
   To develop TLC and HPLC profiles and HPTLC fingerprints and HPLC profiles of the extracts of cashew leaves and testa.

6. **In vitro screening of extract/s and fraction/s for antioxidant activity:**
   To carry out *In vitro* assays in order to evaluate the antioxidant activity of extract/s.

7. **Isolation of phytoconstituents/s:**
   To carry out isolation of phytoconstituents/s from extracts of cashew leaves and testa and confirm its identity and purity.

PART - II

1. **Quantitation of extracts by HPTLC and HPLC:**
   To quantify the content of phytoconstituent/s in extract/s and fraction/s of cashew leaves and testa by optimized HPTLC and HPLC method.

2. **Acute oral toxicity studies:**
   To carry out Acute Oral Toxicity Study in Albino mice following OECD 423 for various extract/s and fraction/s of leaves and testa of cashew.

3. **Evaluation of Antioxidant potential of cashew extracts by various assays on cell lines:**
   To study the effect of cashew extract/s and polyphenol fraction/s and isolated phytoconstituents on the antioxidant defense mechanism of cultured cell lines.
PART-III

1. **Streptozotocin-Nicotinamide Induced model for antidiabetic activity:**
   To carry out preliminary screening of extract/s and fraction/s of cashew leaves, and testa by STZ - Nicotinamide induced rat model for type 2 diabetes.

2. **Evaluation of antidiabetic activity by neonatal induced Streptozotocin (n-STZ) model of Type 2 diabetes mellitus:**
   Selected bioactive extract/s and fractions which will show significant antidiabetic activity in the STZ- Nicotinamide induced model and antioxidant assays will be evaluated for by neonatal induced streptozotocin diabetic rats (n-STZ) model.

3. **Formulation of bioactive extract/s of cashew in suitable dosage form/s:**
   To formulate bioactive extract/s into suitable dosage form and their evaluation for various parameters like friability, hardness, content uniformity testing, dissolution, disintegration and stability studies etc.

The ethanolic extract of cashew testa demonstrated promising antioxidant activity in 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging assay, superoxide scavenging assay, and deoxyribose oxidation assay (Kamath, 2007). The chemical nature of the polyphenols of cashew kernel testa has been determined by means of 2-dimensional paper chromatography (Mathew, 1970). The protective effect of aqueous extract of leaves against STZ-induced diabetes was evaluated in adult rats treated with 175 mg/kg of the extract per os, twice daily (Kamtchouing, 1998). Rats with STZ-induced type 1 diabetes were treated with the hexane extract of cashew leaves at the dose of 300 mg/kg. The treatment helped in reducing diabetes-induced functional and histological alterations in the kidneys (Tedong, 2006). The effect of hexane extract of leaves at doses of 150 and 300 mg/kg/day administered to pregnant diabetic rats caused a 25% and 74.6% reduction respectively in glycaemia (Tedong, 2007). Oral administration of methanol extract of leaves at doses of 35, 175 and 250 mg/kg significantly reduced blood glucose levels in diabetic rats. These results suggest the hypoglycemic effect of the methanol extract of cashew in STZ- induced diabetic rats (Sokeng, 2007).

Literature survey reveals few reports for the anti-hyperglycemic, hypoglycemic and anti-oxidant potential of various extracts of the leaves and testa of *A. occidentale* Linn. Hence the plant was selected for a systematic and detailed study to explore the anti-diabetic activity of phytoconstituents of leaves and testa using various *in vitro* and *in vivo* parameters.