METHODOLOGY

Plant Material: Fresh, mature fruits of Syzygium cumini and roots of Salacia reticulata were collected during July from Western ghats, Kerlala, India. The plant was identified at the Department of Botany, University of Calicut, Kerala, India. Plant material was dried at shade. Ingredients were crushed and powdered using grinder and passed through sieve number #85.

Characterization of the powdered drug: Ingredients of the formulation are to be characterized pharmacognostically, physicochemically and elementally characterized by microscopy, physical constant values and Energy Dispersive Spectroscopy. Selected parameters for the investigation were anatomical study; powder microscopy; Extractive values i.e. alcohol soluble, water soluble, chloroform soluble, ethyl acetate soluble and petroleum ether soluble; Ash values i.e. total ash, water insoluble ash, acid insoluble ash; moisture content value, foreign matter and elemental content.

Preparation of plant extract: The Syzygium cumini fruits have to be first washed well, remove pulp from the seeds. Seeds to be washed several times with distilled water to remove the traces of pulp from the seeds. The seed has to be dried at room temperature. The kernel of the seeds should be separated from the seed coat. The kernel, seed coat, and whole seed have to be powdered in an electrical grinder separately and stored at 5°C until further use. Extraction should be done using suitable solvents. The root of Salacia reticulata has to be cleaned well and should be dried at room temperature. Mill it by grinder and to be extracted using suitable solvents.

Formulation of Nanosuspensions: The simplest method of preparation of nanosuspensions is micronization by colloid or jet milling 20, which improves the dissolution rate but is not having any effect on saturation solubility. Nanosuspension has to be formulated by using any one of the following engineering processes such as precipitation, high pressure homogenization, emulsion / microemulsion template and milling techniques (media milling / dry co-grinding).

Evaluation of nanosuspension: The characterisation of the nanosuspensions is to be done by analyzing criterias such as colour, odour, presence of impurities etc. The other important characteristics of in-vitro evaluations such as particle size and size distribution, particle charge
(Zeta Potential), crystalline state and morphology, saturation solubility and dissolution velocity, stability and in-vivo evaluations are to be done.

**Pharmacological action analysis by in-vitro studies:** The in-vitro antidiabetic activity will be assessed by evaluating α amylase and α glucosidase inhibitory action.

**Pharmacological action analysis by in-vivo studies:**

Animals used: Male albino rats of the Wistar strain weighing around 160–180 g have to be used.

Acute toxicity studies: Animals will be treated with a dose of 200 mg/kg and will be observed individually at least once during the first 30 min, after dosing periodically during the first 24 hrs and up to 14 days after drug administration (according to OECD guidelines).

Experimental induction of diabetes: The animals will be fasted for 18 hours, and diabetes has to be induced by a single intravenous injection of a freshly prepared solution of Streptozotocin (STZ) (55 mg/kg of body weight) in 0.1 M citrate buffer (pH 4.5). The animals will be allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats have to be injected with citrate buffer alone. At 24 hours after STZ injection their fasting blood glucose levels to be estimated. STZ treated animals will be considered as diabetic when the fasting blood glucose levels observed was above 250 mg/dL.

Experimental design: The rats will be divided into two sets each comprising six groups (6 in each group), one for the analysis of biochemical parameters and the other for the evaluation of glucose. Glucose tolerance test has to be performed. All the blood samples should be collected with potassium oxalate and sodium fluoride solution for the estimation of glucose.

**Statistical analysis:** All the grouped data will be statistically evaluated, and the significance of various treatments to be calculated using adequate statistical analysis.

**Comparison of activity:** Comparison of the pharmacologic activity of the formulations to be performed.

**WORK PLAN**

The present study is based on the following plans,
1. Identification and collection of the plant
2. Authentication of the plant by the expert.
3. Drying of the plant and milling.
5. Formulation of the polyherbal formulation churna.
6. Preparation of plant extract.
7. Formulation of nanosuspension.
8. Evaluation of nanosuspension.
9. Incompatibility and stability studies.
11. Statistical analysis of the results.
12. Comparison of the pharmacological activity of the two formulations.