

was planned to screen the leaves, bark and fruit of *F. amplissima* for new porcine pancreatic α -amylase and α -glucosidase inhibitors using *in vitro* assays, which may offer a new, effective and safe therapeutic approach in the management of diabetes.

6.2 MATERIALS AND METHODS

6.2.1 PLANT COLLECTION

Fresh plant materials were collected during the month of October 2009 from Sathyamangalam, Erode district, in Tamil Nadu, India. The taxonomic identity of the plant was confirmed by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu (copy enclosed) and a voucher specimen (No: 006147) was deposited at the Botany Department Herbarium at Bharathiar University, Coimbatore, Tamil Nadu. The plant materials were washed under running tap water to remove the surface pollutants; and the different parts, such as leaves, bark and fruit, were separated mechanically. The separated plant parts were air-dried under shade. The dried samples were powdered and used for further studies.

6.2.2 EXTRACTION OF PLANT MATERIAL

The powdered plant materials, such as leaves, bark and fruit, were packed in small thimbles separately and extracted successively with different solvents, such as petroleum ether, chloroform, acetone and methanol, in an increasing order of polarity using a Soxhlet apparatus. Before extraction with the next solvent, the thimble was air dried each time. Finally, the material was macerated using hot water with constant stirring for 24 h and the water extract was filtered. The different solvent extracts were concentrated by a rotary vacuum evaporator and then air-dried. The dried extract obtained with each solvent was weighed. The percentage of yield was calculated in terms of the air-dried weight of plant material.

The leaf (8.38%) and fruit (7.49%) methanol extract showed higher recovery percentage over other solvent extracts. The stock solution of the extract obtained was prepared (1 mg/mL of respective organic solvents) and used for further analysis.

6.2.3 *IN VITRO* ANTIDIABETIC ACTIVITY

6.2.3.1 Inhibition Assay for α -Glucosidase Activity

α -Glucosidase (0.075 units) was premixed with the extract at various concentrations (50–200 μ g/mL). A substrate of 3 mM *p*-nitrophenyl glucopyranoside (pNPG) was added to the reaction mixture to start the reaction (Miller 1959). The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of Na₂CO₃. The α -glucosidase activity was determined by measuring the *p*-nitrophenol release from pNPG at 400 nm. The IC₅₀ value was defined as the concentration of α -glucosidase inhibitor to inhibit 50% of its activity under the assay conditions.