Optimized Extraction and Characterization of Phytochemicals from Selected Ethnomedicinal Plants for Antidiabetic Phytopharmaceuticals

ABSTRACT

Diabetes mellitus is one of the fast-growing lethal epidemics in India and across the globe. It is responsible for a number of ailments such as vision loss, renal failure, cardiovascular diseases and rise in mortality rate. Due to globalization and rapid urbanization, the cost of synthetic drugs for treating diabetes is also increasing day by day and becomes a financial burden for the people living in developing countries. In comparison to synthetic drugs, herbal drugs have shown much better improvement, less or no side effects, less expensive and are compatible with human physiology as compared to modern synthetic drugs specially in the case of diabetes mellitus. Indigenous and ethnomedicinal plants are gaining popularity and become matter of extensive research among scientific community because of its active role in treatment of acute and chronic diseases among tribal or ethnic communities since a very long time but not validated experimentally or clinically. A significant gap has been identified between the pharmacological application and scientific validation of ethnomedicine. But herbal drugs themselves are facing the problem of low bioavailability, efficacy and safety issue. Therefore, the assigned research work aimed to investigate the antidiabetic and cytotoxic activities of leaf extracts of ethnomedicinal plants *Carica papaya, Cajanus cajan, Coccinia grandis* and *Enhydra fluctuans* and development of phytonanoformulations. The *in silico* analysis revealed that the bioactive phytoconstituents have inhibitory effect on the postprandial key enzymes, α-amylase and α-glucosidase involved in pathophysiology of diabetes mellitus. The molecular docking studies revealed their binding affinities in the ranges -3.7 to -9.3 kcal/mol showed high inhibition potential for the key enzymes. The optimization performed by response surface methodology with box Behnken design gives optimum instrumental conditions for high yielding of phytocompounds for all the four plants. The optimized condition for *C. papaya* was found to be 61°C at a microwave power level of 807 W and 45 min of processing. The optimized conditions for *C. cajan* was found to be 700 W of power, 60 °C of temperature, and 40 min of processing time. The temperature of 55°C, time of 45 min, and power of 763 W was found to be optimized condition for *C. grandis*. Furthermore, the optimized condition for *E. fluctuans* was found to be at a temperature of 58°C, a microwave power level of 803 W, and a processing time of 41 min. Furthermore, preliminary screening, TLC and column chromatography and analytical techniques like LC-MS were used to purify and identify the bioactive phytoconstituents, respectively. The major phytocompounds were phenolics and apigenin, cajanin, camptothecin, ellagic acid, ferulic acid, kaempferol, luteolin, quercetin was identified with their specific retention time. The plant extracts have shown high enzymes inhibiting activities for α-amylase and α-glucosidase enzymes. The inhibitory activity for *C. papaya* (α-amylase: IC_{50}= 40.03 ± 2.61 µg/mL, α-glucosidase: IC_{50}=66.52 ± 1.52 µg/mL), *C. cajan* (α-amylase: IC_{50}= 46.49 ± 2.2 µg/mL, α-glucosidase: IC_{50}=59.51 ± 3.57µg/mL), *C. grandis* (α-amylase: IC_{50}= 52.40±2.68 µg/mL, α-glucosidase: IC_{50}= 76.49±3.97µg/mL) and *E. fluctuans* (α-amylase: IC_{50}= 63.98±3.56 µg/mL, α-glucosidase: IC_{50}= 94.17±7.57 µg/mL) were found to be significant with corresponding standard antidiabetic drug acarbose. The hemolytic activity of all the plant extracts were found to be less

than 6% which was compatible with ISO standards for the intervention of biomaterials. The MTT assay for all the plant extracts were demonstrated low toxicity at the concentration of up to 62.5 µg/mL suggested that toxicity worked in a concentration-dependent manner. The CAM assays of all the three plants except *C. papaya* were found to be safe at all concentrations. Therefore, the concentration-dependent *in vitro, ex vivo* and hemocompatibility cytotoxicity assays revealed the safe use of plant extracts (*C. cajan, C. grandis* and *E. fluctuans*) as potential drug compounds. The plant extracts were mixed in ratio of *in vitro* percentage inhibition activity and an optimized herbal drug combination was prepared in the ratio (50:30:20) based their corresponding percentage inhibition. Furthermore, the herbal drug was loaded into lecithin based liposome synthesized by thin film hydration method. The calculation of encapsulation efficiency of drug loaded liposome, its characterization and cytotoxic evaluation were carried out by UV-Visible spectroscopy, FTIR spectroscopy, DLS, FESEM and optical microscopy. The average size of liposomal formulations with highest encapsulation efficiency was found to be in the range 100-400 nm. The research findings will offer excellent prospects for researchers and experts in the pharmaceutical and ethnomedicinal fields to develop antidiabetic phytopharmaceuticals.

Keywords: Diabetes mellitus, Phytochemicals, Molecular docking, Pharmacokinetics, Optimization, Phytochemical screening, Purification, Characterization, Enzyme inhibition Hemocompatibility, Cytotoxicity, *ex vivo* studies, drug optimization, liposome, encapsulation