ABSTRACT

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterised by abnormally elevated blood glucose levels (hyperglycemia). Diabetes Mellitus is caused by insufficient insulin synthesis or inappropriate insulin usage by the cells (American Diabetes Association). In 2017, more than 425 million people aged 20 to 79 had diabetes, including 72 million Indians; by 2045, this number is expected to rise to 629 million (IDF diabetes atlas - 2017). Hyperglycemia is associated with microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular, cerebrovascular, and peripheral artery disease) problems. In addition, hyperglycemic situations are reported to damage smaller and larger blood vessels. α-glucosidase inhibitors (α-GI) are used for treating Type 2-diabetes mellitus (T2-DM). They function by inhibiting the catalytic activity of α-glucosidase, slowing the glucose release rate from oligosaccharides. Thereby reducing the rate of glucose absorption in the small intestine. Thus, α-GIs are used primarily to treat postprandial hyperglycemia, are the first-line treatment for T2-DM and are recommended as first-line drugs for pre-diabetic conditions.

Natural products have been used since ancient times and are used by the current society as they are potential resources. With the future rise of the diabetic population and the limitations of the available drugs, it becomes a meaningful task to identify new phytomolecules with α-glucosidase inhibitory activity. Plants are a rich source of bioactive chemicals that display decisive pharmacological actions. α-GIs compounds are ubiquitously present in the plants. This study explored the potential of plants belonging to diverse families as α-GIs using a combinatorial approach.

Objective 1: The preliminary step was to establish a physical library of plant materials collected across different sites in India. In this objective, a high throughput screening of the plant extracts has been carried out to analyze their potential to inhibit α- glucosidase. This has

helped to validate the existing literature of various plants reported for α-glucosidase inhibition and find new plants with their potential to inhibit the enzyme. Among 420 crude extracts from 70 plants, 174 extracts had IC_{50} concentration of ≤ 3 mg/ml, which was dominated by the hexane extract. *Terminalia arjuna* – bark (Arjuna) (IC_{50} ≤ 0.001 to 1.23 mg/ml), *Eucalyptus tereticornis* – leaves (forest red gum) (IC_{50} 0.001 to 2.18 mg/ml), *Artocarpus heterophyllus* – bark (jackfruit) (IC_{50} 0.005 to 1.15 mg/ml), *Curcuma longa* – rhizome bark (turmeric) (IC_{50} ≤ 0.001 to 0.082 mg/ml), *Tribulus terrestris* – fruit (devil's weed) (IC_{50} 0.12 to 1.11 mg/ml), and *Cassia anugustifolia* - leaves (senna) (IC_{50} 0.045 to 3.06 mg/ml) were categorised based on the presence of α-glucosidase inhibitory plant secondary metabolite (PSM) in all six solvents. It suggested that these plants may have a broad range of PSM with different polarities that inhibit α-glucosidase at low concentrations in crude/purified form. For the plants like *Murraya koenigii*- leaves (kadi patta), *Swertia chirayita*- whole plant (chirayata), *Withania coagulans*- fruit (Punir dodi), *Dillenia indica*- fruit (elephant apple) only their hexane extracts showed IC_{50} values < 1 mg/ml. The others solvent extracts of these plants did not show any inhibition.

Objective 2: *In-silico* studies were conducted to understand the possible role of structurally diverse PSM in inhibiting α- glucosidase activity. A virtual PSM library of 2752 molecules from 50 shortlisted α-glucosidase inhibitor plants selected from the first objective was prepared. Further, they were subjected to molecular docking against α-glucosidase. The structural activity relationship studies revealed that among the top 100 molecules with the least binding energy, 34% belonged to sesquiterpenoids. Through ADMET and pkCSM studies, 539 molecules were found to have a drug-like properties. For the first time on in silico basis, this study reports the following molecules, Withanolide M, 17 β-hydroxyl withanolide K (17BHWK), Withanolide J, Coagulansins A and B, Withasomniferol C, Withacoagulin I, 1, 8-Dihydroxy-3-carboxy-9, 10-anthraquinone (DHCA), and Swerilactone C as a potential α-glucosidase inhibitors which can be studied further.

Objective 3: Based on the literature and our experimental data *Withania coagulans* was selected to isolate bioactive compound for α- glucosidase inhibition. Only the hexane extract (2% yield) showed α- glucosidase inhibitory properties with IC_{50} of 0.013 mg/ml and Ki of 0.012 mg/ml. The purification strategy of the bioactive fraction was designed, and at the final purification stage, the bioactive fraction S-IV-F3 showed IC_{50} of 0.004 mg/ml and Ki of 0.0037 mg/ml. The characterization and structure elucidation studies showed that the bioactive fraction consisted of olefinic fatty acids and fatty acid methyl esters. The NMR analysis also supports the presence of fatty acid-like compounds in the S-IV-F3.

Objective 4: *Withania coagulans* fruit extract has shown a better α- glucosidase inhibiting activity than the standard drug acarbose. At various stages of its purification, it has shown increasing activity, thus inferring that it has immense potential to be used as an α-glucosidase inhibitor. So, it becomes essential to understand the additional benefits it may provide to individuals consuming it to manage DM. *Withania coagulans* bioactive fractions were analysed for their antioxidants, anti-microbial, and cytotoxic activities. Enzyme kinetics studies revealed the non-competitive inhibition nature of bioactive fraction against α- glucosidase. This is mainly due to the type of compounds present in the extracts and the molecules binding to the allosteric site of the enzyme, thus reducing the affinity of the enzyme towards the substrate from interacting with the active site of the enzyme. Among the five bacterial strains tested *S. aureus* was found to be highly sensitive to S-I with IC_{50} of 4 mg/ml, followed by *B. cereus* (8 mg/ml), *K. pneumoniae* (25 mg/ml), *P. aeruginosa* (25 mg/ml), and *E. coli* (32 mg/ml). However, S-I showed lower antioxidant activity. Consumption of *W. coagulans* extract may provide additional benefits apart from diabetes management. The purification strategy and the reported molecules in this study have shown an immense potential to reduce cytotoxicity and may be represented as a potential venture for drug molecules.

Objective 5: As the bioactive fraction (S-I and S-IV-F3) of *W. coagulans* showed non-competitive inhibition under *in-vitro* conditions, evaluating the PSMs for their interaction with the enzyme under in-silico conditions becomes interesting. In this objective, a library of known PSMs was prepared through GC-MS analysis of WC bioactive fractions. These PSMs were subjected to blind ensemble docking with the enzyme to evaluate the allosteric binding affinity to different allosteric pockets of the enzyme. The molecular dynamics studies used the Enzyme-PSM binary structure and Enzyme-PSMs-Isomaltose ternary structure. The molecular dynamic simulation (MDS) studies of selected molecules showed that Methyl 12,13-tetradecadienoate (MTDD) was consistently found to bind the allosteric site at pocket 3 throughout the simulation period. Further, binding to the allosteric site of the enzyme, MTDD reduced the affinity of the enzyme towards isomaltose, evidencing its non-competitive nature of enzyme inhibition.