α-Amylase Inhibitory Secondary Metabolites from *Artemisia pallens* Wall ex DC – Biochemical and Docking Studies

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Background : Diabetes Mellitus Type-2 (DM-2) has become a challenging disease worldwide as many young adults are also getting affected by it due to sedentary lifestyle and wrong diets. Synthetic drugs are widely used for its treatment but they have negative side effects on humans. Hence search for new anti-hyperglycaemic molecules of plant origin is still on. We used leaf & bud material of *Artemisia pallens* Wall ex DC for isolation of non-polar secondary metabolites which were then tested for their porcine pancreatic alpha amylase (PPA) inhibitory activity. The effective, PPA inhibitory acetone extract was characterized by LC-MS/MS for it's secondary metabolite content. Molecular docking was then used to find out the binding energies of ten best secondary metabolites with respect to the prescribed drug: Acarbose. Binding energies better than Acarbose were obtained.

Methodology

Plant Material Collection & Extraction

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Qualitative & Quantitative Estimation of Phenols, Flavonoids & Terpenoids

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Preliminary Starch-Iodide Assay

Quantitative DNSA Assay

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LC-MS?MS Analysis of Acetone Leaf Extract

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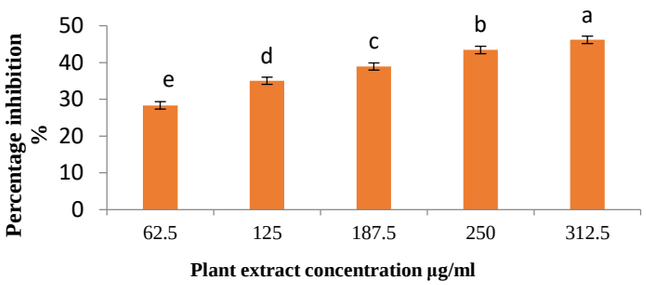
Molecular Docking Studies

Results

Table 1.Estimation of Phenols, Flavonoids & Terpenoids in *A. pallens* Acetone Bud Extract

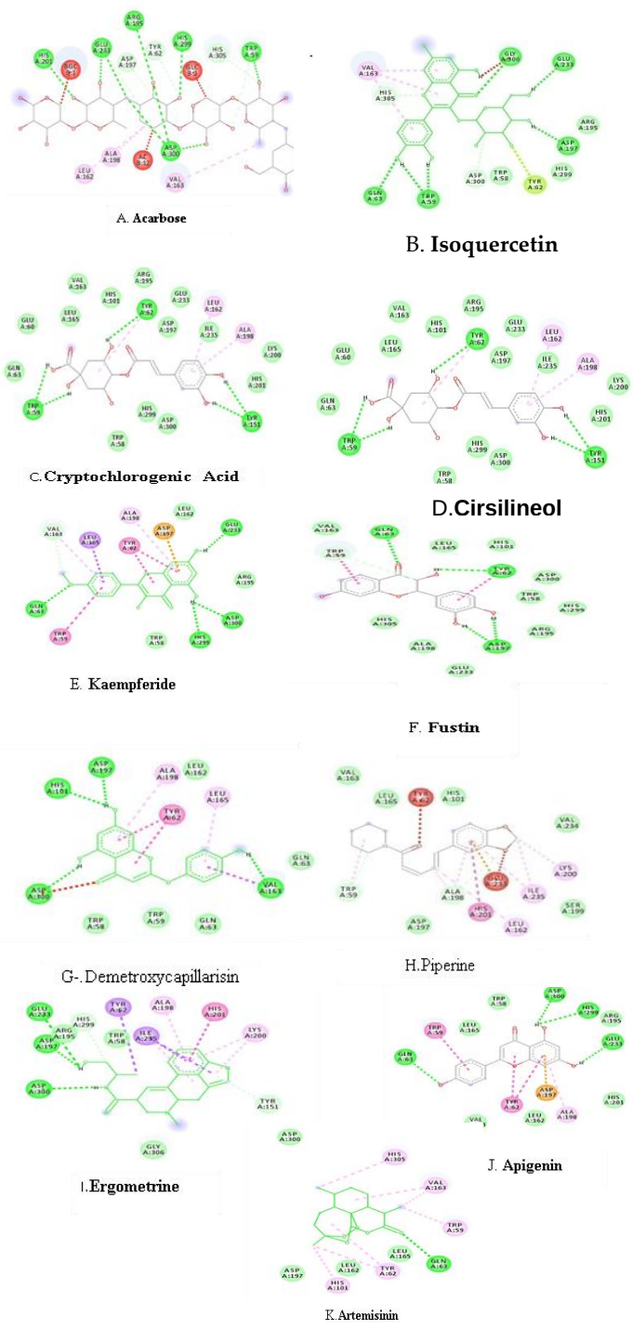
Total Phenols	Total Flavonoids	Total Terpenoids
9.1 ±0.004 µg/mg	275.5±0.01 µg/mg	68.5 ±0.01 µg/mg

Figure 1. Percent Inhibition of PPA with Different Conc. of Acetone Bud Extract



IC_{50} value for acetone bud extract was 338.05 µg/ml

Figure 2. Representation of PPA Protein-Ligand Interactions in 2-D.



Conclusions

- A rapid workflow involving Biochemical Amylase Inhibition Assay, LC-MS/MS Analysis and Molecular Docking was established for phytochemicals extracted from *A. pallens*.
- Ten best phytochemicals showed good amylase inhibition biochemically as well as with molecular docking (Fig. 2).
- They interacted with the important catalytic residues Asp197, Glu233 and Asp300 of PPA through hydrogen bonds, van der Waal forces, and pi-pi interactions.

References

- Sudha P., Zinjarde S.S., Bhargava S.Y., Kumar A.R. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine. 2011 Dec;11(1):1-0.*11*, 5, doi:10.1186/1472-6882-11-5.
- Miller G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959 Mar 1;31(3):426-428.https://doi.org/10.1021/ac60147a030.
- Baba S.A., Malik S.A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. Journal of Taibah University for Science. 2015 Oct 1;9(4): 449-454, https://DOI.org/10.1016/j.jtusci.2014.11.001.

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