Flavonoids from *Argyreia nervosa* (Burm.f) Bojer: A Ready Arsenal Against Pests as well as Diabetes

Anuja D. Kamble[1] and Anjali A. Kulkarni[1]*

[1]Department of Botany, Savitribai Phule Pune University, Ganeshkhind Road, Pune 411 007, Maharashtra, India.

*Corresponding author: anjali.uop@gmail.com or akulkarni@unipune.ac.in

INTRODUCTION

•Diabetes mellitus -Type 2 (DM 2) has currently become one of the most challenging non-infectious diseases to treat.

•Despite the introduction of several oral non-insulin drugs to treat NIDDM, patient compliance remains poor due to severe side effects. Hence search for new molecules with reduced side effects is still continuing.

•In recent years, *in-silico* methodologies have become an important aspect of drug discovery process due to their time saving and cost effective nature. These computer aided drug design (CADD) approaches are advantageous for reducing the use of animals for *in vivo* experiments.

•Many *in-silico* studies have been proposed to prove the potency of natural products to inhibit the alpha amylase and alpha glucosidase enzymes.

•The current study was carried out to identify and validate the alpha amylase inhibitory flavonoids from *Argyreia nervosa* (Berm.f.) Bojer.

OBJECTIVE

We aimed to find out if flavonoids from leaf extracts of *Argyreia nervosa* (Burm.f) Bojer (Family: Convolvulaceae) could exhibit alpha-amylase inhibitory activity *in vitro* and *in silico*.

MATERIALS AND METHODS

•The leaf sample was collected from Savitribai Phule Pune University and authenticated by Botanical Survey of India (BSI/WRC/IDEN.CER./2020/94)

•Soxhlet Extraction of 50 grams of leaf sample was done using solvents with increasing polarity.

•Preliminary phytochemical analysis was done using freshly prepared extracts.

•The concentrated extracts (1mg/ml DMSO) were checked for alpha-amylase inhibitory activity using Starch-Iodide assay.

•Total flavonoid content of chloroform extract was estimated by aluminium chloride method.

•Column chromatography was performed to separate the different compounds present in the chloroform extract.

•LC-MS analysis of the alpha amylase inhibitory fraction obtained from column chromatography was done to identify the compounds present in it.

•The compounds identified by LC-MS analysis were docked on porcine pancreatic alpha amylase PDB ID: 1OSE (AutoDock 4.2.6)

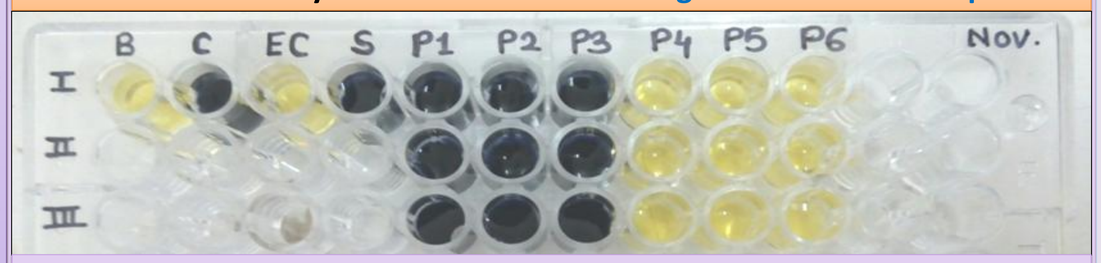
•The identified flavonoids were also screened for Lipnskie's Rule of five.

RESULTS

Table 1: Phytochemical analysis of chloroform leaf extract.

Test	Chloroform Leaf Extract
Alkaloids (Mayer's)	- - -
Alkaloids (Dragendorff's)	- - -
Alkaloids (Wagner's)	- - -
Alkaloids (Hager's)	- - -
Phenols	+ + +
Flavonoids	+ + +
Tannins	- - -
Terpenes	+ + +
Steroids	- - -
Quinones	+ + +

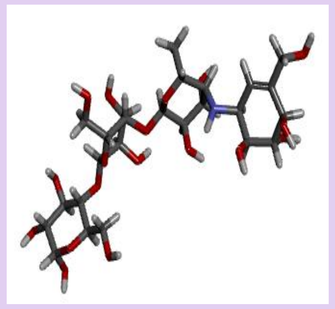
Figure 1 : PPA Inhibitory Activity of Various Plant Extracts Using Qualitative Starch Iodide Assay. Blue wells contain strong PPA Inhibitor from plant.



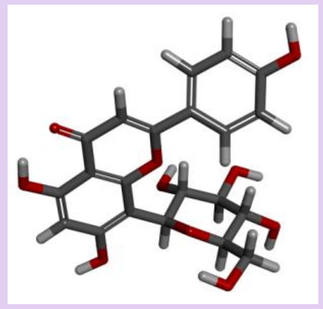
B: Blank, C: Control, EC: Enzyme Control, SC: Substrate (drug) Control, P1: Pet Ether, P2: Chloroform, P3: Ethyl Acetate, P4: Acetone, P5: Methanol, P6: Water

Flavonoid content : 378.83 ± 0.76 µg/mg

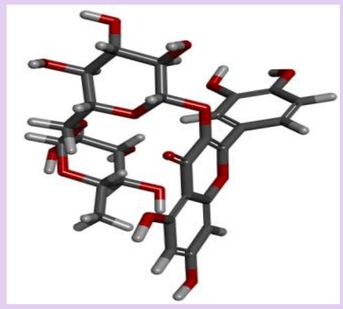
Figure 2 :The 3D ligand structures used in docking studies for targeting alpha-amylase enzyme (Identified by LC-MS)



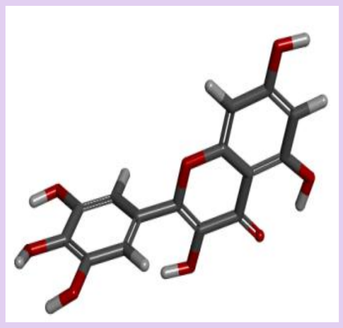
Acarbose
(PubChem ID : 41774)



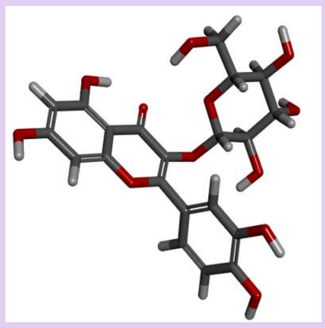
Vitexin
(PubChem ID : 5280441)



Rutin
(PubChem ID : 5280805)



Myricetin
(PubChem ID : 5281672)



Isoquercetin
(PubChem ID : 5280804)

Figure 3 :The comparative graph of binding energy of ligands and Acarbose

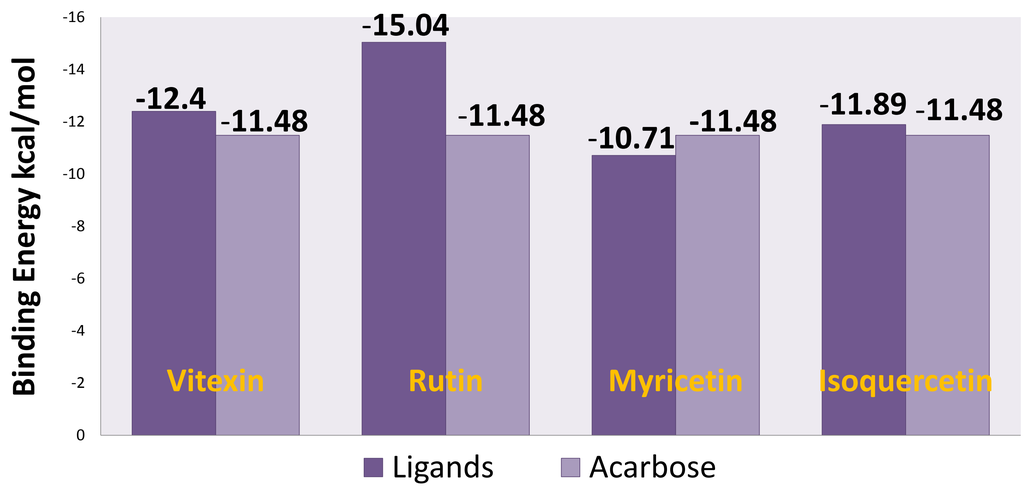
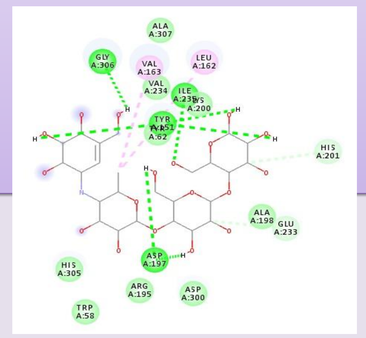
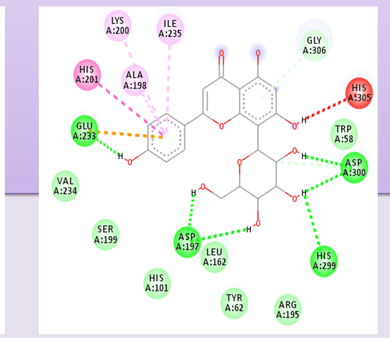


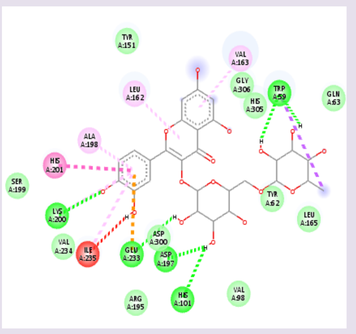
Figure 4: 2-D representation of different types of intermolecular protein-ligand interactions.



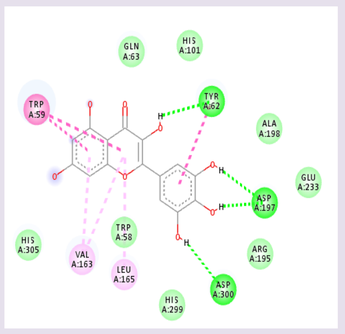
Acarbose
(PubChem ID : 41774)



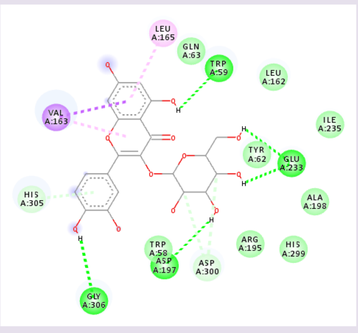
Vitexin
(PubChem ID : 5280441)



Rutin
(PubChem ID : 5280805)



Myricetin
(PubChem ID : 5281672)



Isoquercetin
(PubChem ID : 5280804)

Table 2: Physicochemical parameters of ligands screened for Lipinski Rule.

Ligand	Mol. Wt. g/mol	H- Bond Donor	H-Bond Accep-tor	LogP	Molar Refractivity	Rules obeyed
Vitexin	432.4	7	10	-0.0655	103.5340	4/5
Rutin	610.5	10	16	-1.8788	137.4954	2/5
Myricetin	318.23	6	8	1.7165	75.71527	4/5
Isoquercetin	464.4	8	12	-0.7306	106.2738	3/5

CONCLUSION

•Out of diverse secondary metabolites identified fwith LC-MS-MS from chloroform extract of *Argyreia nervosa* (Burm.f) Bojer. , vitexin, rutin, myricetin and isoquercetin showed significant alpha amylase inhibitory activity.

•ASP197 was the essential amino residue involved in the intermolecular interactions.

•This work provides important insights towards drug discovery process for controlling diabetes mellitus.

REFERENCES

•Trease, GE; Evans WC. Pharmacognosy, 15th Ed.; Saunders Publishers, London, 2002 pp. 42-44, 221-229, 246-249, 304-306, 331-332, 391-393.

•P S; Zinjarde SS; Bhargava SY; Kumar AR. Potent a-amylase inhibitory activity of Indian Ayurvedic medicinal plants, *BMC Complement Altern Med* 11, 5 2011, https://doi.org/10.1186/1472-6882-11-5

•Lipinski CA; Lombardo F; Dominy BW; Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev, 2001, 46, 3–26, DOI : 10.1016/s0169-409x(00)00129-0.