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Comparative Anti- diabetic studies and Molecular docking of Amorphophallus paeoniifolius (Dennst.) Nicolson and Colocasia esculenta (L.) Schott

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ABSTRACT

In the present investigation anti – diabetic activity of ethanol extract of *Amorphophallus paeoniifolius* and *Colocasia esculenta* was evaluated. The anti – diabetic activity of ethanol extract of *Amorphophallus paeoniifolius* and *Colocasia esculenta* was evaluated by Alpha-amylase inhibition assay and Alpha glucosidase inhibition assay.In these tuber crops, Alpha amylase activity with IC_{50} value of standard is 0.69216±0.314, IC_{50} of *Amorphophallus paeoniifolius* is 1.5088±0.114, IC_{50} of *Colacasia esculenta* is 3.7537±0.112 and Alpha glucosidase activity with IC_{50} of standard is 1.56401±0.211 µg/ml, IC_{50} of *Amorphophallus paeoniifolius* is 3.657±0.144 µg/ml, IC_{50} of *Colacasia esculenta* is 4.461±0.144 µg/ml. In conclusion, from the results of present study it is confirmed that anti – diabetic activity of ethanol extract of *Amorphophallus paeoniifolius* may contribute anti- diabetic potential.Molecular docking is a computational method used to predict the preferred orientation of one molecule (ligand) to another molecule (protein or receptor) when they are bound together. In the context of tubers, molecular docking can be used to study the interactions between bioactive compounds and proteins involved in various biological processes. Betulinic acid, lupeol, quercetin glycoside, stigmasterol are the most important anti – diabetic compounds in *Amorphophallus paeoniifolius.*

KEYWORDS: IC_{50} value, Alpha- amylase inhibition assay, Alpha glucosidase inhibition assay, anti- diabetic potential, molecular docking

1. INTRODUCTION

According to the Indian medicinal system most of the plants have medicinal properties. Diabetes is a long-lasting metabolic conditioncategorized by high blood sugar levels. It affects millions of people worldwide, causing serious health complications if left unmanaged. Anti-diabetic measures are essential in controlling blood sugar levels, preventing complications, and improving the quality of life for individuals with diabeteslevels (World Health Organization. (2019)

Lifestyle modifications are crucial in managing diabetes. A healthy diet, regular physical activity, and weight management are essential in controlling blood sugar levels. A diet rich in fiber, fruits, and vegetables, and low in sugar, salt, and unhealthy fats can help regulate blood sugar (American Diabetes Association2022). Regular physical activity, such as walking, running, or swimming, can improve insulin sensitivity and glucose uptake. Maintaining a

healthy weight through a combination of diet and exercise can also improve insulin sensitivity and reduce the risk of complications (National Institute of Diabetes and Digestive and Kidney Diseases. (2022).

In addition to lifestyle modifications, medications and therapies play a vital role in managing diabetes. Alternative and complementary therapies, such as herbal supplements, acupuncture, and yoga, may also be beneficial in managing diabetes. Certain herbal supplements, such as berberine, cinnamon, and fenugreek, have been shown to have anti - diabetic properties. Otherwise, some of the tuber crops shows anti – diabetic properties.

Tuber crops are also grown as intercrop of Coconut. *Amorphophallus paeoniifolius* is native to tropical Asia, including India, Sri Lanka, and Southeast Asia. It is Found in forests, woodlands, and grasslands. Prefers well-drained soil and partial shade. Cultivated for its edible corm, which is rich in starch and fiber. Used as a food source in many Asian cultures.*Colocasia esculenta* is a perennial herb with a large, fleshy corm. Can be grown as an ornamental plant in tropical and subtropical regions. Used as a food source in many tropical cultures.Both of them have many medicinal properties. Especially, they have anti-diabetic property.

Molecular docking is a computational method used to predict the preferred orientation of one molecule (ligand) to another molecule (protein or receptor) when they are bound together.

2. METHODOLOGY

The corms were collected from the tribal area, Channa Vila (Vithura). Local information was collected through conversations with “Moopan” the Village head and through elder peoples. This study investigates the traditional values of some tuberous plants from study area and linked 26 important tuberous plants used as drug for diabetes. Among the 26 tuberous plants, two tubers similar as *Amorphophallus paeonifollius* (Dennst.) Nicolson and *Colocasia esculenta* (L.) Schott were named for farther studies. These two plants are generally used for diabetes by Thiruvananthapuram District ethnical people. Phytochemical and proximate analysis are already done. So, both of them are entering into practical section for anti – diabetic study.

The anti – diabetic activity of ethanol extract of *Amorphophallus paeoniifolius* and *Colocasia esculenta* was evaluated by Alpha- amylase and Alpha glucosidase inhibition assay.

ANTI – DIABETIC ACTIVITY

Alpha Amylase Inhibition Assay

Procedure:

Porcine pancreatic α- amylase (PPA; A05329G191; 1 mL) was liquified in 9 mlof 20 mM phosphate buffer (pH 6.9) to give 4 Unit/ MI results. The stock solution of the plant extracts was prepared by a standard value of 1µg/ ml. From this different attention were prepared. Potato starch (0.5 w/ v) was dissolved in 20 mM phosphate softened saline (pH 6.9) and placed in a scorching water bath to get a clear solution. 40 µL of plant extract, 160 µL of

distilled water, and 400 µL of starch solution were mixed. The response started by the addition of 200 µL. of the enzyme result and the tubes were incubated at 25 ° C for 3 min at room temperature. The enzyme solution was added at 1 min interval from the launch of the response. 200 µL of the admixture was withdrawn into a separate test tube containing 100 µL. of DNS color reagent (50.68 g sodium potassium tartrate dissolved in 70 mL of 2 M NaOH with 0.026 mM A of 3,5- dinitro salicylic acid) and placed in a water bath maintained at 85- 90 ° C for 15 min. The admixture in each tube was adulterated with 900 mL of distilled water and the absorbance was measured at 540 nm. For each attention of the excerpt used, blank incubation was prepared by replace the enzyme result with distilled water (200 µL). Control incubations were carried out in an analogous manner by replacing factory excerpt with 40 µL of 2 DMSO. All the tests were run in triplet. From the value attained, the chance (w/ v) of maltose generated was calculated from the equation attained from the maltose standard estimation wind (0- 0.1 w/ v maltose). The position of inhibition was calculated as follows.

Inhibition () 100 response (at t = 3 min), where response = mean maltose in sample 100/ mean maltose in control.

Alpha Glucosidase Inhibition Assay

Materials:

- Sodium phosphate buffer- pH6.8
- Alpha glucosidase enzyme
- PNPG
- Acarbose

Procedure:

Both the control and excerpts were treated with 100mM phosphate buffer of pH 6.8.0.1 U/ ml of glucosidase enzyme and 1.25 mM PNPG. The excerpts were taken in an attention of 10µg/ ml and made up in a final volume of 200µL at 37 °C. The response in presence of Acarbose was used as the positive control. The response was initiated by adding PNPG and the released PNPG was determined by measuring absorbance at 410nm using spectrophotometer after 10 minutes of response time. Background readings were excluded by abating the absorbance of admixture without enzyme. The absorbance is directly commensurable to the enzymatic activity.

$$\text{Enzyme inhibition \%} = \frac{(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{reaction}})\ 100\%}{\text{Absorbance}_{\text{control}}}$$

DOCKING

Protein preparation:

The 3D structure of the Free fatty acid receptor 1 (GPR40) protein, with a resolution of 2.33 Å (PDB code: 4PHU), was retrieved from the Protein Data Bank (PDB). Chain A was selected for docking studies.

Active site determination:

The active site of the protein was determined using the PDBSum online tool. Active site residues: TYR91, ARG183, TYR2240 and ARG2258.

Molecular docking:

The flexible docking procedure proposed by Trott and Olson was used to carry out the molecular docking with a few minor adjustments. As a result, Python Prescription 0.8 (PyRx 0.8), a package that incorporates Auto Dock Vina, was used to conduct the docking analysis of the identified chemicals with our chosen proteins. For the proteins, partial charge and atom type (PDBQT) files have been created (with their earlier formed PDB files as inputs). All of the ligand's bonds were allowed to rotate freely, which made the receptor stiff. Except for the grid box, which was altered in accordance with the active sites of protein molecules, many other parameters were retained at their default values. After the molecular docking investigations, ten combinations for each protein-ligand complex for all ligands were constructed and the top ligands with the least binding energy were further analyzed (Dallakyan and Olson *et al*., 2015). The protein-ligand interaction was studied using Pymol and Discovery studio visualizer.

Pharmacokinetic properties

ADMET properties of the ligand is analyzed using pkCSM online server (https://biosig.lab.uq.edu.au/pkcsm/). An ADMET study assesses the pharmacokinetics of a drug which stands for Absorption, Distribution, Metabolism, Excretion, and Toxicity. The prediction of the fate of a drug and the effects caused by a drug inside the body, such as how much drug is absorbed if administered orally and how much is absorbed in the gastrointestinal tract, is an indispensable part of drug discovery. Similarly, if the absorption is poor, its distribution and metabolism would be affected, which can lead to causing neurotoxicity and nephrotoxicity. Ultimately, the study aims to understand a drug molecule's disposition within an organism. Thus, the ADMET study is one of the most essential parts of computational drug design.

3. RESULT AND DISCUSSION

Anti – diabetic activity

Alpha Amylase Assay

Absorbance of control = 0.996 nm

IC $_{50}$ of standard = 0.69216±0.314

IC $_{50}$ of *Amorphophallus paeoniifolius*= 1.5088±0.114

IC $_{50}$ of *Colacasia esculenta* = 3.7537±0.112

Alpha amylase activity with IC_{50} value of standard is 0.69216±0.314, IC_{50} of *Amorphophallus paeoniifolius* is 1.5088±0.114, IC_{50} of *Colacasia esculenta* is 3.7537±0.112.

Based on IC_{50}value*Amorphophallus paeoniifolius*showing more anti – diabetic activity than *Colacasia esculenta.*

Alpha Glycosidase Inhibition Assay

Absorbance of control = 0.998 nm

IC $_{50}$ of standard = 1.56401±0.211 µg/ml

IC $_{50}$ of *Amorphophallus paeoniifolius* = 3.657±0.144 µg/ml

IC $_{50}$ of *Colacasia esculenta* = 4.461±0.144 µg/ml

Alpha glucosidase activity with IC_{50} of standard is 1.56401±0.211 µg/ml, IC_{50} of *Amorphophallus paeoniifolius* is 3.657±0.144 µg/ml, IC_{50} of *Colacasia esculenta* is 4.461±0.144 µg/ml. Based on IC_{50}value*Amorphophallus paeoniifolius*showing more anti – diabetic activity than *Colacasia esculenta.*

Molecular docking:

Interaction of FFAR1 protein with the ligands

In this study (table 1) **stigmasterol**, **betulinic acid**, and **lupeol** demonstrated no hydrogen bonding interactions with the active site residues of the FFAR1 protein, indicating limited binding potential. In contrast, the compound **isoquercetin** formed two significant hydrogen bonds with the active site residues **ARG183** and **SER2247**, suggesting a stronger and more specific interaction. Furthermore, the reference drug **Fasiglifam** exhibited two hydrogen bonds with the residues **TYR2240** and **ARG2258**, highlighting its established binding efficacy. It is to be noted that the compound isoquercetin showed hydrophobic interaction with ARG2258, one of the active site residues of the reference drug. These findings underscore the potential of isoquercetin as a promising ligand, comparable to the reference drug in its interaction with key active site residues.

Pharmacokinetic properties of the hit compounds using pkCSM

In this study (table 2) the compound **isoquercetin** exhibited a strong docking score of **-6.4 kcal/mol**, highlighting its significant binding affinity to the FFAR1 protein. Interaction analysis confirmed that the ligand forms critical interactions with the active site residues **ARG183** and **SER2247**, suggesting its potential as an effective inhibitor of FFAR1. Additionally, the compound displayed favorable ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties without any indications of toxicity. Importantly, it showed good pharmacokinetic properties, reinforcing its suitability as a promising bioactive candidate for therapeutic applications.

4. EXPERIMENTAL SECTION

a. Alpha Amylase Inhibition Assay

Porcine pancreatic α- amylase, phosphate softened saline, phosphate buffer, DNS color reagent, DMSO

b. Alpha Glucosidase Inhibition Assay

Sodium phosphate buffer- pH6.8, Alpha glucosidase enzyme, PNPG, Acarbose

c. Molecular docking

FFAR1 protein, pkCSM

5. CO.NCLUSION

Most of the plants have medicinal properties. Thus the current study provides comprehensive information regarding the anti – dioabetic activities of the two important tubers*Amorphophallus paeoniifolius*and *Colacasia esculenta*. Based on this study, *Amorphophallus paeoniifolius*has more anti – diabetic activities than *Colacasia esculenta.*

Docking revealed that *Amorphophallus paeoniifolius*had more anti – diabetic compounds. Betulinic acid, lupeol, quercetin glycoside, stigmasterol are the most important anti – diabetic compounds in *Amorphophallus paeoniifolius.*

ACKNOWLEDGEMENT

The authors extent their heartfelt thanks to local informants of Channa vila tribal area and Dr. Sruthy B, Accuratus Scientia Natural Product Research and Development Pvt Ltd Attingal, for all the laboratory facilities and support.

Competing interests

The authors declare no existing competing interest.

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Figure 1

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Visiting of study area

Figure 2

Alpha Amylase Assay

Absorbance of control = 0.996 nm

Standard for Alpha amylase assay

Concentration(µg/ml)	Average absorbance of std (nm)	Average % inhibition activity of std
0.2	0.289	72.1
0.4	0.247	75.2
0.6	0.215	78.41
0.8	0.198	80.12
1	0.154	84.54

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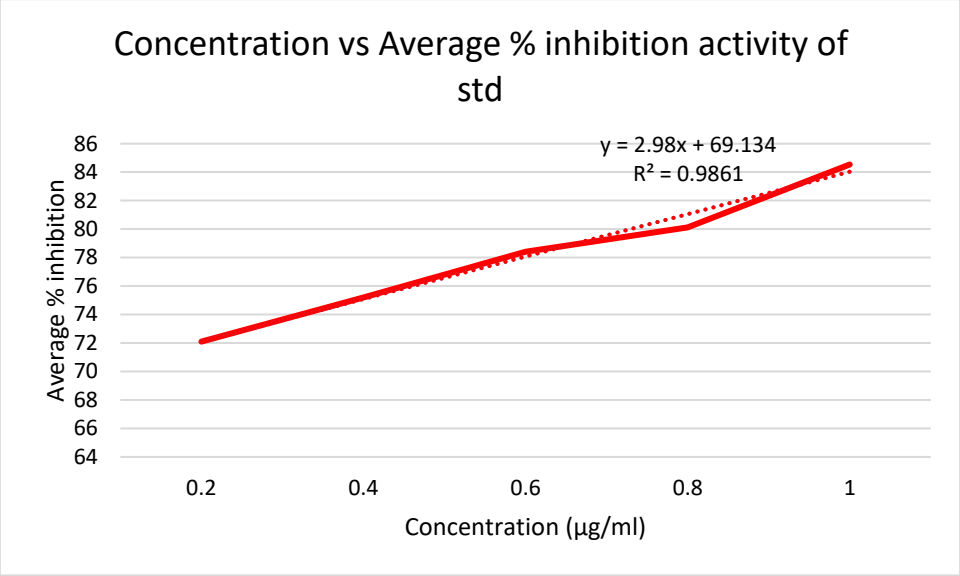

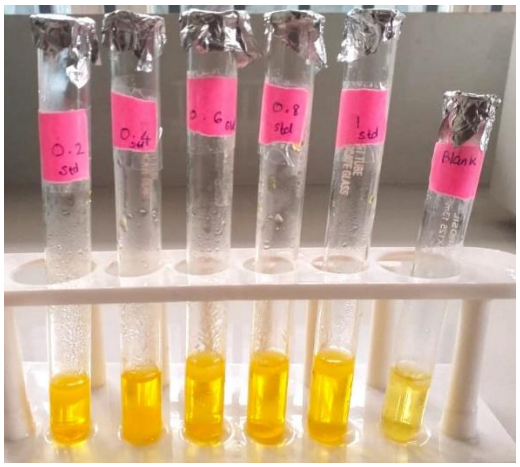

Absorbance of Amorphophallus paeoniifolius

Concentration(µg/ml)	Average absorbance of std (nm)	Average % inhibition activity of std	Average absorbance of *Amorphophallus paeoniifolius* (nm)	Average % inhibition activity of *Amorphophallus paeoniifolius*
0.2	0.289	72.1	0.411	65.21
0.4	0.247	75.2	0.355	64.36
0.6	0.215	78.41	0.364	63.45
0.8	0.198	80.12	0.276	72.29
1	0.154	84.54	0.262	73.69

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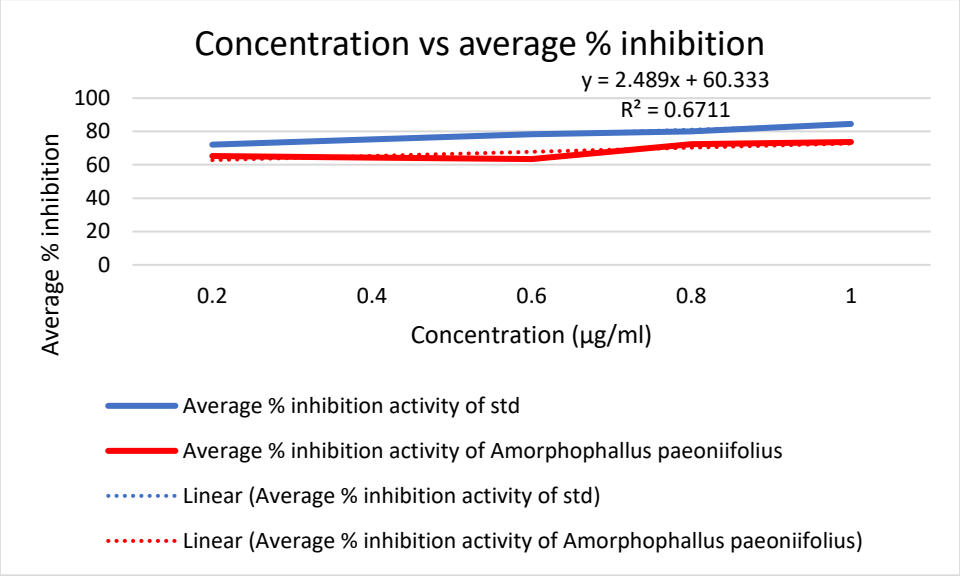
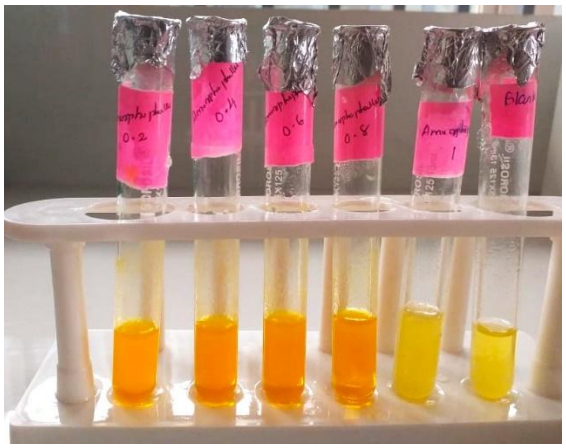
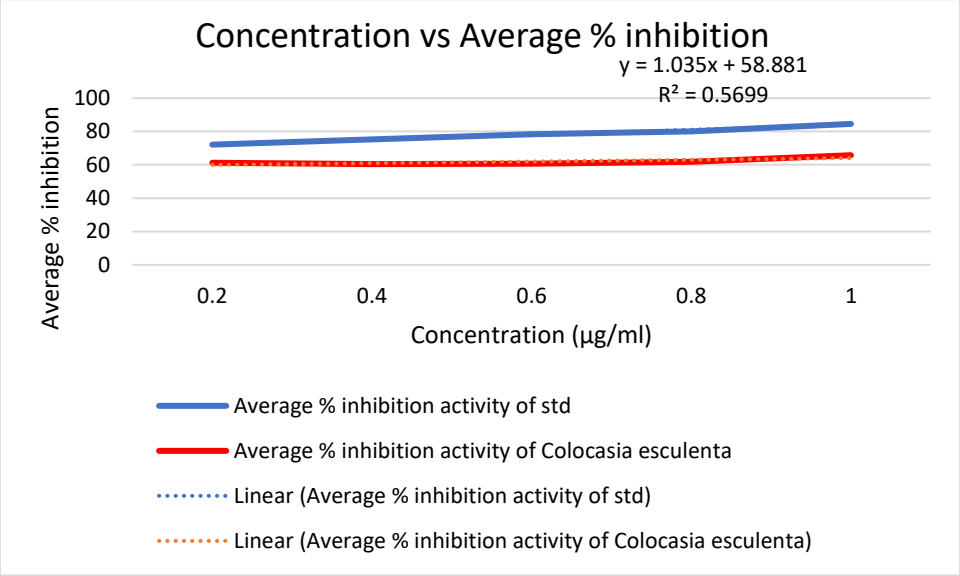
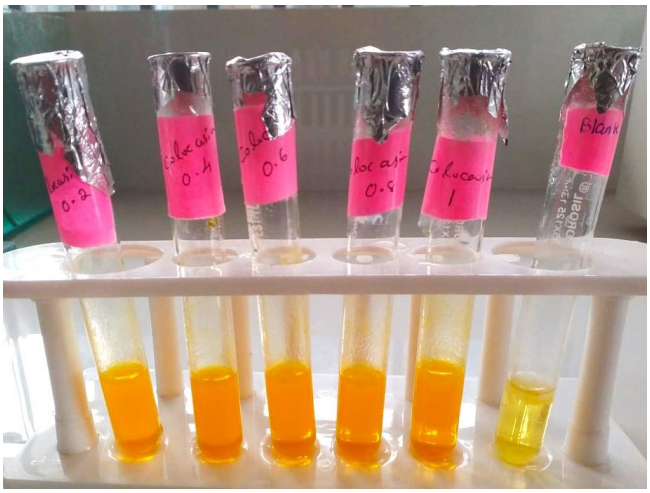




Absorbance of *Colocasia esculenta*

Concentration(µg/ml)	Average absorbance of std (nm)	Average % inhibition activity of std	Average absorbance of *Colocasia esculenta* (nm)	Average % inhibition activity of *Colocasia esculenta*
0.2	0.289	72.1	0.477	61.24
0.4	0.247	75.2	0.394	60.44
0.6	0.215	78.41	0.391	60.74
0.8	0.198	80.12	0.384	61.75
1	0.154	84.54	0.341	65.76





IC $_{50}$ of standard = 0.69216±0.314

IC $_{50}$ of *Amorphophallus paeoniifolius* = 1.5088±0.114

IC $_{50}$ of *Colacasia esculenta* = 3.7537±0.112

Figure 3

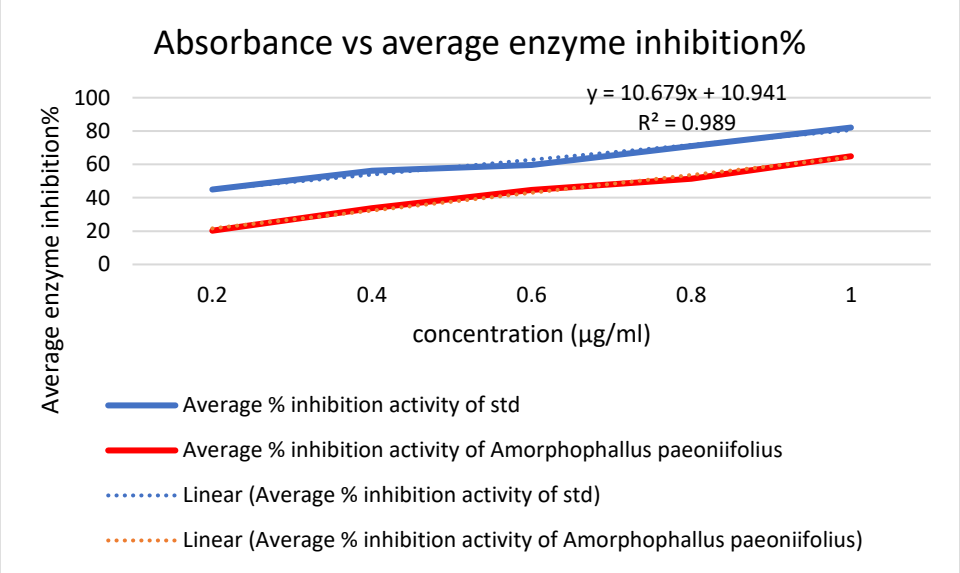
Alpha Glycosidase Inhibition Assay

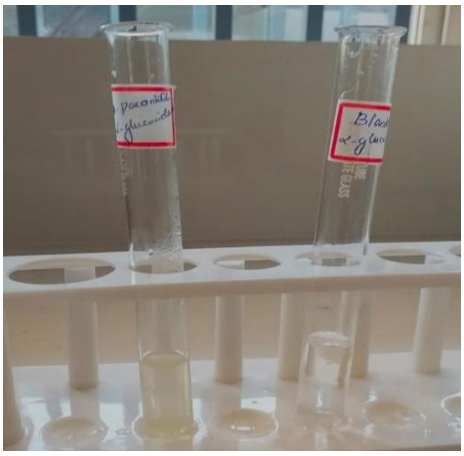
Absorbance of control = 0.998 nm

Absorbance of *Amorphophallus paeoniifolius*

Concentration(µg/ml)	Average absorbance of std (nm)	Average % inhibition activity of std	Average absorbance of *Amorphophallus paeoniifolius* (nm)	Average % inhibition activity of *Amorphophallus paeoniifolius*
0.2	0.544	44.94	0.788	20.24
0.4	0.432	56.28	0.654	33.81
0.6	0.399	59.62	0.547	44.64

0.8	0.287	70.95	0.481	51.32
1	0.177	82.09	0.347	64.88





Absorbance of *Colocasia esculenta*

Concentration(µg/ml)	Average absorbance of std (nm)	Average % inhibition activity of std	Average absorbance of *Colocasia esculenta* (nm)	Average % inhibition activity of *Colocasia esculenta*
0.2	0.544	44.94	0.799	19.13
0.4	0.432	56.28	0.684	30.77
0.6	0.399	59.62	0.544	44.94
0.8	0.287	70.95	0.511	48.28
1	0.177	82.09	0.498	49.6

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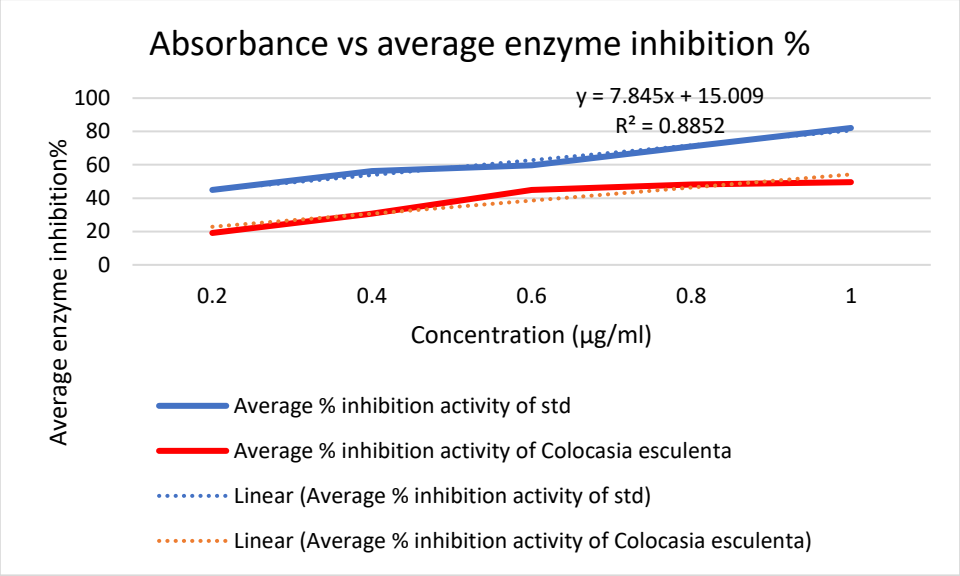
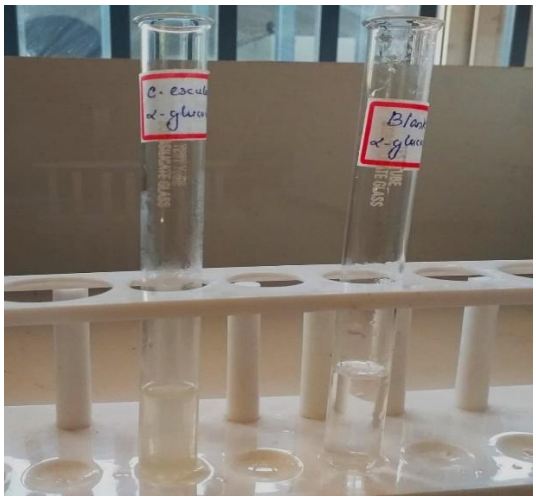




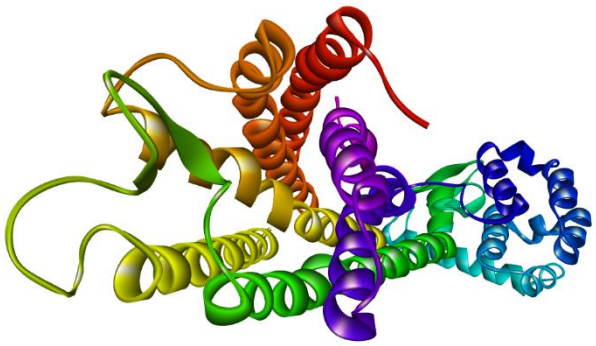
IC $_{50}$ of standard = 1.56401±0.211 µg/ml

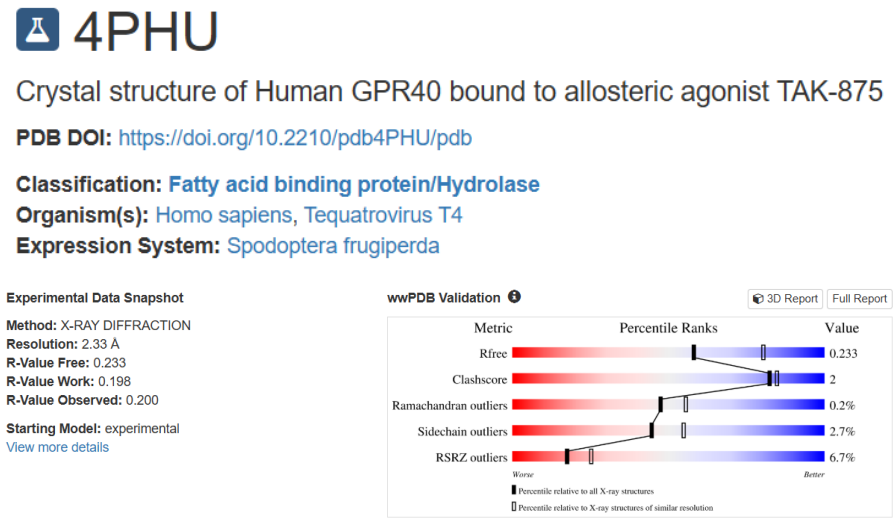
IC $_{50}$ of *Amorphophallus paeoniifolius* = 3.657±0.144 µg/ml

IC $_{50}$ of *Colacasia esculenta* = 4.461±0.144 µg/ml

Molecular docking

Structure of the protein (PDB ID: 4PHU)





Active site residues of FFAR1: TYR91, ARG183, TYR2240, ARG2258

Structure of secondary protein

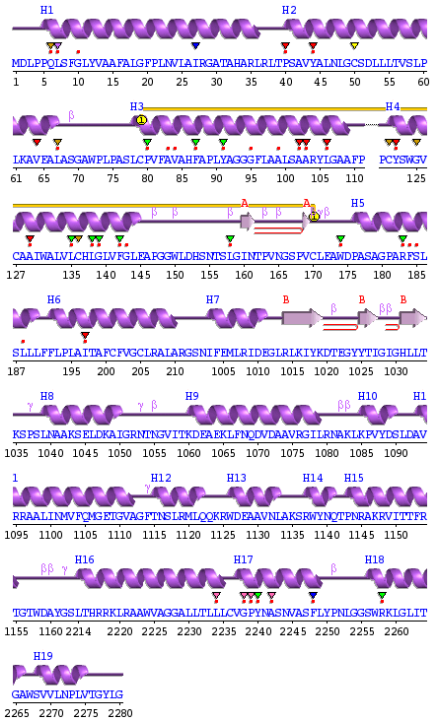


Table 1 :Interaction of FFAR1 protein with the ligands

Phytochemical	Pubchem ID	Binding Affinity (kcal/mol)	Hydrogen Bond	Distance	Hydrophobic interaction
Stigmasterol	5280794	-8.1	Nil	-	PHE 142 , ALA 83 , VAL 84 , LEU 138 , PHE 142
Betulinic Acid	64971	-7.4	Nil	-	ARG 2258

Lupeol	259846	-7.3	Nil	-	VAL 84
Isoquercetin	5280804	-6.4	ARG 183 , SER 2247 ,	1.84 2.27	LYS 2259 , ARG 2258
Fasiglifam (Agonist/native ligand)	24857286	-10.2	TYR 2240 , ARG 2258	2.18 2.62	LEU 138 , PHE 142 , PRO 80 , ALA83 , LEU 171

Table 2: Pharmacokinetic properties of the hit compounds using pkCSM

Property	Model Name	Isoquercetin	Unit
Absorption	Water solubility	-2.925	Numeric (log mol/L)
Absorption	Caco2 permeability	0.242	Numeric (log Papp in 10^{-6} cm/s)
Absorption	Intestinal absorption (human)	47.999	Numeric (% Absorbed)
Absorption	Skin Permeability	-2.735	Numeric (log Kp)
Absorption	P-glycoprotein substrate	Yes	Categorical (Yes/No)
Absorption	P-glycoprotein I inhibitor	No	Categorical (Yes/No)
Absorption	P-glycoprotein II inhibitor	No	Categorical (Yes/No)
Distribution	VDss (human)	1.846	Numeric (log L/kg)
Distribution	Fraction unbound (human)	0.228	Numeric (Fu)
Distribution	BBB permeability	-1.688	Numeric (log BB)
Distribution	CNS permeability	-4.093	Numeric (log PS)
Metabolism	CYP2D6 substrate	No	Categorical (Yes/No)
Metabolism	CYP3A4 substrate	No	Categorical (Yes/No)
Metabolism	CYP1A2 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2C19 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2C9 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP2D6 inhibitior	No	Categorical (Yes/No)
Metabolism	CYP3A4 inhibitior	No	Categorical (Yes/No)
Excretion	Total Clearance	0.394	Numeric (log ml/min/kg)
Excretion	Renal OCT2 substrate	No	Categorical (Yes/No)
Toxicity	AMES toxicity	No	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	0.569	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	No	Categorical (Yes/No)
Toxicity	hERG II inhibitor	Yes	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	2.541	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	4.417	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	No	Categorical (Yes/No)
Toxicity	Skin Sensitisation	No	Categorical (Yes/No)
Toxicity	*T.Pyriformis* toxicity	0.285	Numeric (log ug/L)
Toxicity	Minnow toxicity	8.061	Numeric (log mM)

ISSN PRINT 2319 1775 Online 2320 7876
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3D interaction of the lead compound isoquercetin with the target protein

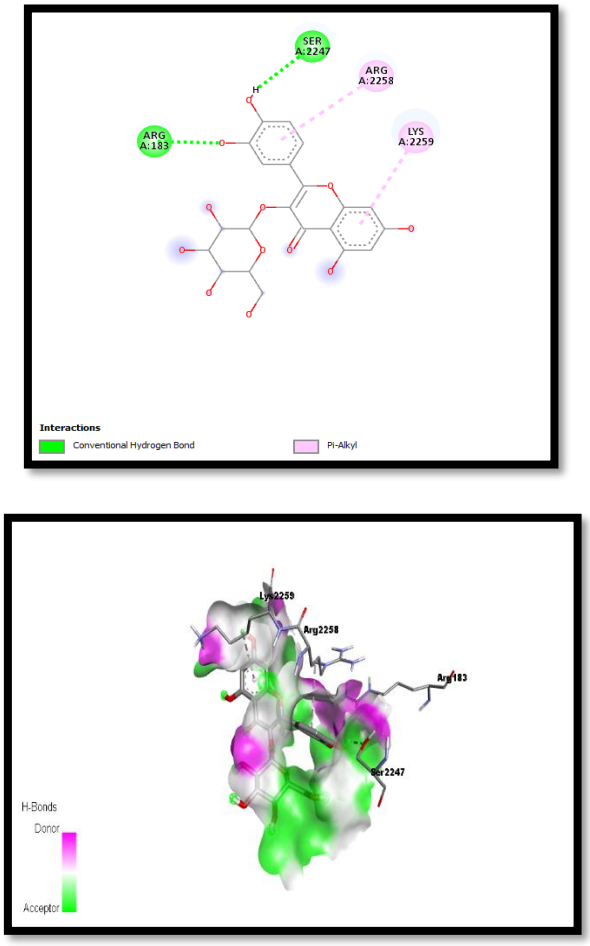
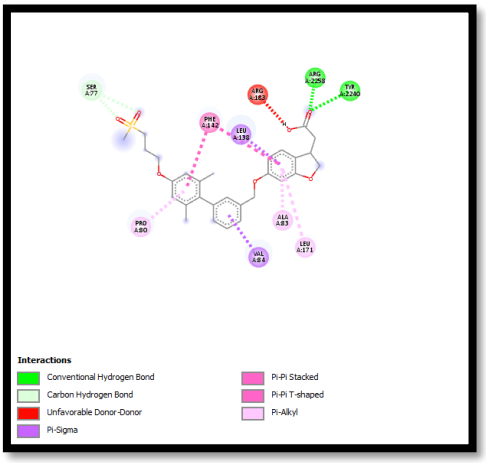


Figure: Docking between the selected protein and the ligand Isoquercetin a) 2D view b) 3D view

(A)



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(B)

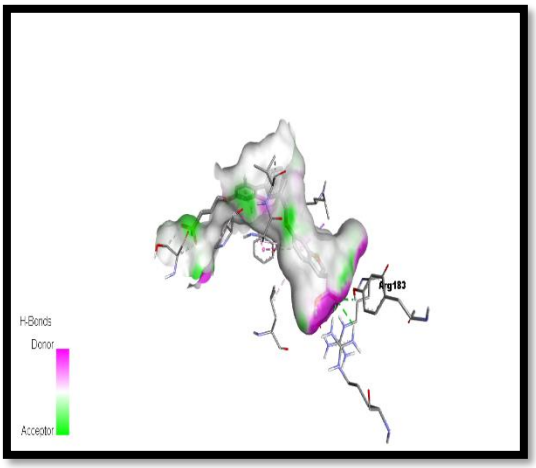

Figure: Docking between the selected protein and the reference drug a) 2D view b) 3D view