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SHORT COMMUNICATION

In Vitro Alpha Amylase and Protein Denaturation Inhibitory Activities of *Premna barbata* Extracts with *In Silico* Insights

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Abstract

Background: Around the world, medicinal plants are an important part of many traditional medical systems. Different species of *Premna* showed diversified bioactivities such as anticancer, antimicrobial, antidiabetic, etc. *Premna barbata* Wall. Ex Schauer, commonly known as *Aganyo* and *Bakhara* in Uttarakhand, India, is a member of the Lamiaceae family. *Premna barbata* has not been explored much. **Aim:** This manuscript evaluated chloroform and ethyl acetate extracts of *P. barbata* with alpha amylase and cyclooxygenase-2 inhibition, with *in silico* insights. **Methods:** Both extracts were phytochemically screened, which confirmed the presence of protein, carbohydrate, glycoside, and flavonoid. Then, chloroform and ethyl acetate extracts were biologically tested by *in vitro* alpha amylase and protein denaturation inhibitory methods associated with Diabetes mellitus, and inflammation. Molecular docking analyses were performed using *P. barbata* phytoconstituents (geniposidic acid, epi-alpha-bisabolol, 1-octen-3-ol) with alpha amylase and cyclooxygenase-2 receptors. **Results:** *Premna barbata* chloroform extract, *P. barbata* ethyl acetate extract, and acarbose showed alpha amylase inhibitory properties with IC_{50} values of 67.85µg/ml, 70.37µg/ml, and 67.35µg/ml, respectively. *Premna barbata* chloroform, *P. barbata* ethyl acetate extract, and aspirin showed good protein denaturation inhibition with IC_{50} values of 72.85µg/ml, 74.18µg/ml, and 26.76µg/ml, respectively. Molecular docking analysis data showed that geniposidic acid and epi-alpha-bisabolol observed with good docking scores against alpha amylase and cyclooxygenase-2 receptors. These phytoconstituents may be responsible for the antidiabetic and antiinflammatory properties of *P. barbata*. **Conclusion:** These findings confirmed that *Premna barbata* showed good potential as an antidiabetic and anti-inflammatory agent.

Major Findings: *Premna barbata* showed significant antidiabetic and anti-inflammatory properties. Computational investigation confirmed the importance of geniposidic acid and epi-alpha-bisabolol in the treatment of Diabetes mellitus and inflammation.

Keywords: Alpha Amylase, Extraction, Molecular Docking, *Premna barbata*, Protein Denaturation

1. Introduction

Premna barbata Wall. Ex-Schauer (*P. barbata*) belongs to the Lamiaceae family. *Aganyo* and *Bakhara* are its other names, commonly used in Uttarakhand, India, and Ayurveda[1]. The plant contains various glycosides, mainly iridoid glycosides, alkaloids, flavonoids, terpenoids, volatile oil, polysaccharides, and fatty acids[2,3]. *Premna latifolia* and *Premna tomentosa* have already been observed with cytotoxic activities. A previous study by our group described the antiproliferative potency of *P. barbata* extract against different cancer cell lines[4,5]. Along with anticancer activity, *Premna spinosa* showed antihyperglycemic and hypolipidemic properties in an animal model. Roots of *P. latifolia* Roxb, leaves of *Premna serratifolia* L. and methanolic extract of *Premna herbacea* Roxb exhibit remarkable antidiabetic activities by

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streptozotocin-nicotinamide induced diabetic rat model, alpha-glucosidase inhibition process, and high-fat diet induced animal model, respectively[6]. However, *P. barbata* is still unexplored in the treatment of Diabetes mellitus. Also, chronic inflammation directly leads to type 2 Diabetes mellitus and metabolic disorders[7]. Hence, the current study focused on exploring the antidiabetic and anti-inflammatory potentials of *P. barbata* using *in vitro* and *in silico* alpha amylase and protein denaturation inhibitory activities[8].

2. Materials and Methods

2.1 Plant Material

The collection of plant samples was done from Jones Estate village near Nainital district of Uttarakhand, India (29.355 N, 79.543 W and 1450 m altitude) and authenticated. Plant samples were shade dried below 40°C, and ground coarsely[9].

2.2 Plant Extraction Process

P. barbata, dried leaves were extracted with petroleum ether in a Soxhlet's apparatus. Then petroleum ether extract was concentrated using a rotary evaporator and transferred to a previously weighed china dish followed by drying in vacuum desiccator. Then the defatted marc was air dried followed by extracted with chloroform and ethyl acetate. The yield of the extracts was calculated and stored in amber colored bottle[10].

2.3 Phytochemical Screening

P. barbata Chloroform (PBC) and *P. barbata* Ethyl Acetate (PBEE) extracts were phytochemically screened by the standard method[11].

2.4 *In Vitro* Alpha Amylase Inhibition of *P. barbata* Extracts

Final dilutions (10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, and 80µg/ml) of *P. barbata* chloroform (PBC) and PBEE extracts were prepared. Reference acarbose solutions (10µg/ml, 20µg/ml, 40µg/ml, 60µg/ml, and 80µg/ml) were prepared using the same diluent. In a microtiter plate, phosphate buffer (pH: 6.8) (50 µl100 mM), alpha amylase (10µl 2 Unit/mL), and 20µl of different dilutions of plant and acarbose were mixed well and incubated at 37°C. 1% soluble starch (20µl) was added and incubated at 37 °C. Dinitrosalicylic acid (100µl) was added tothe solution and boiled for 10 minutes. The absorbance of the resulting mixture was taken. At 540 nm, the absorbance of the resulting solution was measured using a UV-Visible spectrophotometer (Shimadzu UV-1900i)[12].

$$\%\ alpha\ amylase\ inhibitory\ activity = \left[1 - \frac{Absorbance\ of\ Sample}{Absorbance\ of\ Control}\right]$$

2.5 Protein Denaturation Assay of *P. barbata* Extracts

About 1.0g of aspirin was measured using a Metler Toledo microanalytical balance (ME204) and dissolved in 10ml water as solvent (100 µg/ml). The final dilutions of aspirin (10, 20, 40, 60, and 80) µg/ml were prepared with the same diluent. Final dilutions (10, 20, 40, 60, and 80) µg/ml of *P. barbata* chloroform (PBC) and PBEE extracts were used in the assay[13]. In a series of test tubes, phosphate buffer (pH 6.4, 2.8ml), 0.2ml of egg albumin, and 2ml of different concentrations of PBC, PBEE extracts, and aspirin were taken and mixed well. Here, distilled water was considered a negative control. Then the reaction mixtures were incubated at 37°C for 30 minutes, and kept at 70°C for 10 minutes, followed by cooling at room temperature for 10 minutes. Absorbance of the reaction mixture before and after denaturation was measured for each concentration. Absorbance of the reaction mixture was measured at 680 nm by an Ultra Violet (UV)-Visible Spectrophotometer (Shimadzu UV-1900i)[14].

$$\%\ Protein\ Denaturation\ inhibition = \left[\frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control}\right]$$

2.6 Molecular Docking Studies of *P. barbata* Phytoconstituents with Alpha Amylase and Cyclooxygenase-2 Receptors

As per the literature review, the leaf of *P. barbata* consisted of three main phytoconstituents, such as 1-Octen-3-ol, epi-alpha-bisabolol and geniposidic acid. 2QV4 and 6COX receptors were selected for molecular docking studies targeting alpha amylase and cyclooxygenase, respectively. 2QV4 receptor is a human pancreatic alpha-amylase enzyme with acarbose as a

complexed ligand. The receptor comprises 496 amino acids[15]. Acarbose interacted with ARG 195, ASP 300, HIS 299, TYR 62, GLN 63, LEU 162, ARG 195, GLU 233, LEU 165, TRP 59, HIS 101, VAL 107, ILE 51, ALA 106, ASN 105, GLY 164, and THR 163. 6COX receptor belongs to cyclooxygenase-2 receptor complexed with 1-Phenylsulfonamide-3-Trifluoromethyl-5-ParabromophenylPyrazole. 6COX receptor complexed ligand interacted with ARG 120, GLN 192, LEU 352, SER 353, GLY 526, TRP 387, TYR 385, LEU 384, PHE 381, ALA 527, PHE 518, VAL 523, LEU 359, LEU 531, VAL 349, TYR 355, and ARG 513. The structures of 1-Octen-3-ol, epi-alpha-bisabolol and geniposidic acid were drawn and energy minimized by Avogadro software. The ligands and receptors were saved in PDBQT format. The grid box sizes of 2QV4 and 6COX receptors were centre_x = 12.21, centre_y = 56.848, and centre_z = 20.137 and centre_x = 23.404, centre_y = 26.553, centre_z = 48.353 with size_x = 24, size_y = 24, size_z = 24, and exhaustiveness = 8. Autodock vina, MGLTools and Drug Discovery Studio visualizer were used for a molecular docking study[16].

3. Results and Discussion

3.1 Phytochemical Screening and Extraction Yield

The percentage yield values (%w/w) of PBC, PBEE extracts were 32.25% and 28.45%, respectively. Phytochemical analysis confirmed the presence of protein, carbohydrate, glycoside, and flavonoid (Table 1)[17].

3.2 *In Vitro* Alpha Amylase Inhibition Data of *P. barbata* Extracts

The *in vitro* alpha amylase inhibitory assay data showed that IC_{50} values of PBC, PBEE and acarbose were 67.85µg/ml, 70.37µg/ml, and 67.35µg/ml, respectively (Figure 1). Chloroform extract showed good alpha amylase inhibition compared to standard acarbose[18].

3.3 Protein Denaturation Inhibition Data of *P. barbata* Extracts

The *in vitro* protein denaturation inhibitory assay data showed that IC_{50} values of PBC, PBEE, and aspirin were 72.85µg/ml, 74.18µg/ml, and 26.76µg/ml, respectively

Table 1. Phytochemical analysis of *P. barbata* extracts

Constituents	Tests	*P. barbata*Chloroform Extract (PBC)	*P. barbata*Ethyl Acetate Extract (PBEE)
Amino acids	Ninhydrin test	N	N
Proteins	Biuret test	P	P
	Millon's test	P	P
Carbo hydrates	Molisch's test	P	P
	Fehling's test	P	P
Steroids	Salkowski test	N	N
Triterpenoids	Salkowski test	N	N
Alkaloids	Mayer's test	N	N
	Dragendroff's test	N	N
	Hager's test	N	N
Glycoside	Sodium hydroxide reagent test	P	P
	Keller kiliani Test	P	P
	Borntrager's test	P	P
	Trim-Hill reagent test	P	P
	Foam test	P	P
Flavonoids	Shinoda test	P	P
Tannin and Phenolic compounds	Ferric chloride test	N	N
	Lead acetate test	N	N

P: Positive; N:Negative

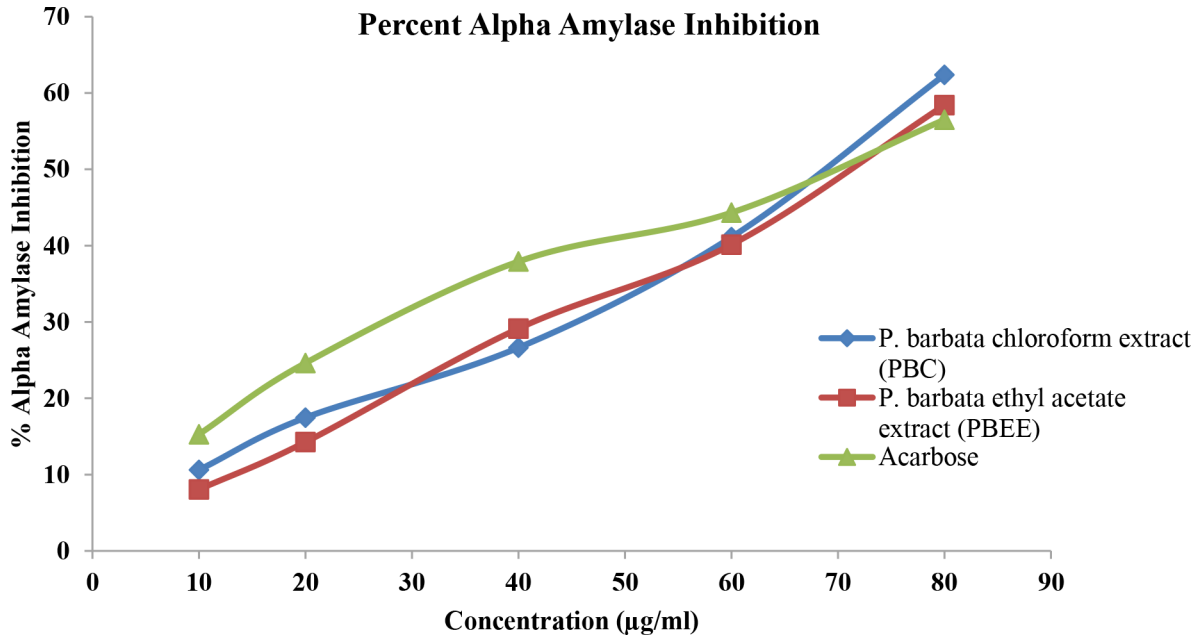


Figure 1. *In vitro*alpha amylase inhibition data of *P. barbata*extracts.

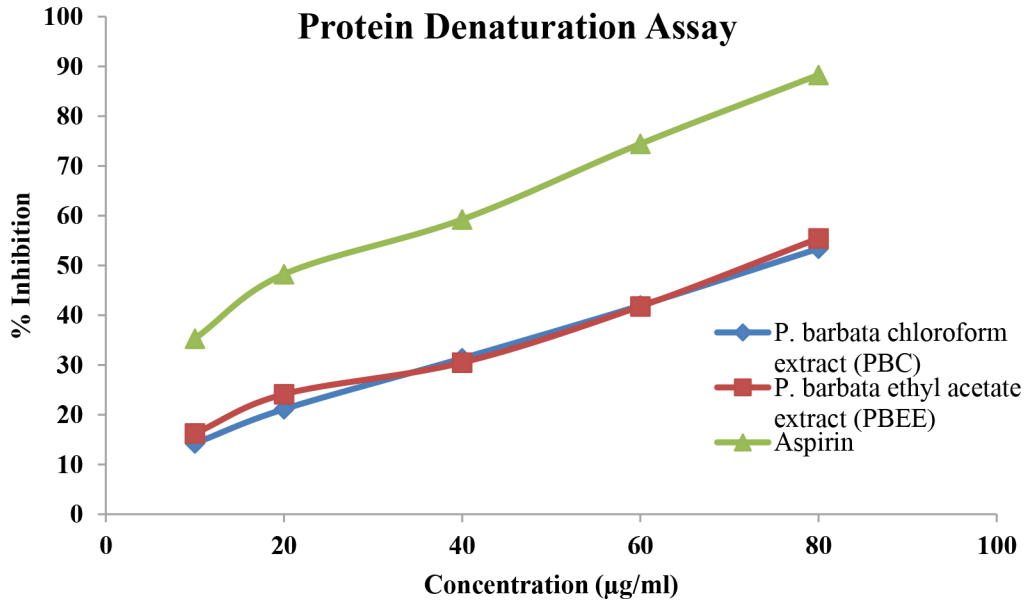


Figure 2. Protein denaturation inhibition data of *P. barbata* extracts.

(Figure 2). Chloroform extract showed good protein denaturation inhibition compared to standard aspirin[19].

3.4 Molecular Docking Interaction Data of *P. barbata* Phytoconstituents with Alpha Amylase and Cyclooxygenase-2 Receptors

Molecular docking analysis data of *P. barbata* phytoconstituents with alpha amylase (2QV4) and inflammation-oriented receptor (6COX) showed that dock scores of epi-alpha-bisabolol (-7.2 kcal/mol), geniposidic acid (-7.8 kcal/mol), 1-octen-3-ol (-4.7 kcal/mol), and epi-alpha-bisabolol (-7.1 kcal/mol), geniposidic acid (-6.7 kcal/mol), 1-octen-3-ol (-5.3 kcal/mol), respectively. Epi-alpha-bisabolol interacted with 2QV4 receptor via TRP 58, TYR 62, LEU 162, ALA 198, LYS 200, HIS 201, ILE 235 by pi-alkyl interaction; and ASP 300, VAL 234, TYR 151,

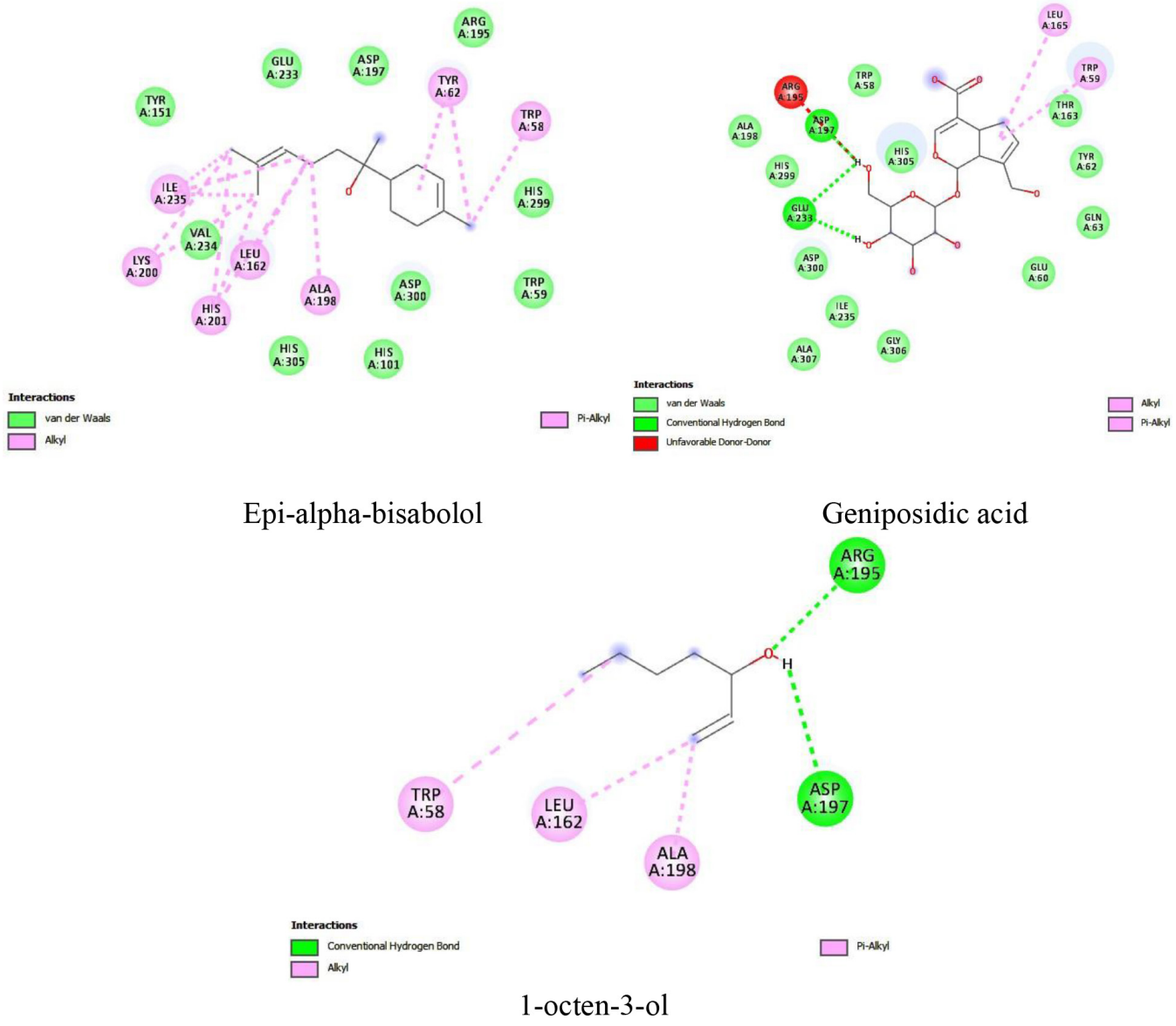


Figure 3. Molecular docking interactions data of Epi-alpha-bisabolol, Geniposidic acid, and 1-octen-3-ol with 2QV4.

HIS 299, and ASP 197 by van der waal interactions. Geniposidic acid interacted with 2QV4 via ASP 197, GLU 233 by hydrogen bond interaction; TRP 59, LEU 165 by pi-alkyl interaction; and THR 163, TYR 62, HIS 305, HIS 299, ASP 300, and ALA 198 by van der waal interactions. 1-octen-3-ol interacted with 2QV4 via ARG 195, ASP 197 by hydrogen bond interaction; TRP 58, LEU 162, ALA 198 by pi-alkyl interaction (Figure 3). Geniposidic acid showed the best dock score of (-) 7.8 kcal/mol with the alpha amylase receptor (PDB: 2QV4). Epi-alpha-bisabolol showed the best dock score of (-) 7.1 kcal/mol with cyclooxygenase-2 receptor (PDB: 6COX)[20].

Epi-alpha-bisabolol interacted with 6COX receptor via GLY 526, SER 530, TYR 348, ARG 120, SER 353, MET 522 by van der waal interactions; and ALA 527, TYR 385, LEU 352, VAL 523, VAL 349, TYR 355, LEU 351, LEU 359, VAL 116 by pi-alkyl interaction. Geniposidic acid interacted with 6COX via GLN 192, SER 579 by hydrogen bond interaction; ASN 581, GLN 350, GLU 346, and PHE 580 by van der waal interactions. 1-octen-3-ol interacted with 6COX via ARG 376 by hydrogen bond interaction; ILE 124, PHE 381, PHE 209, PHE 529 by pi-alkyl interactions; and ASN 375, ILE 377, GLY 227, VAL 228, and GLY 533 by van der waal interactions (Figure 4)[21].

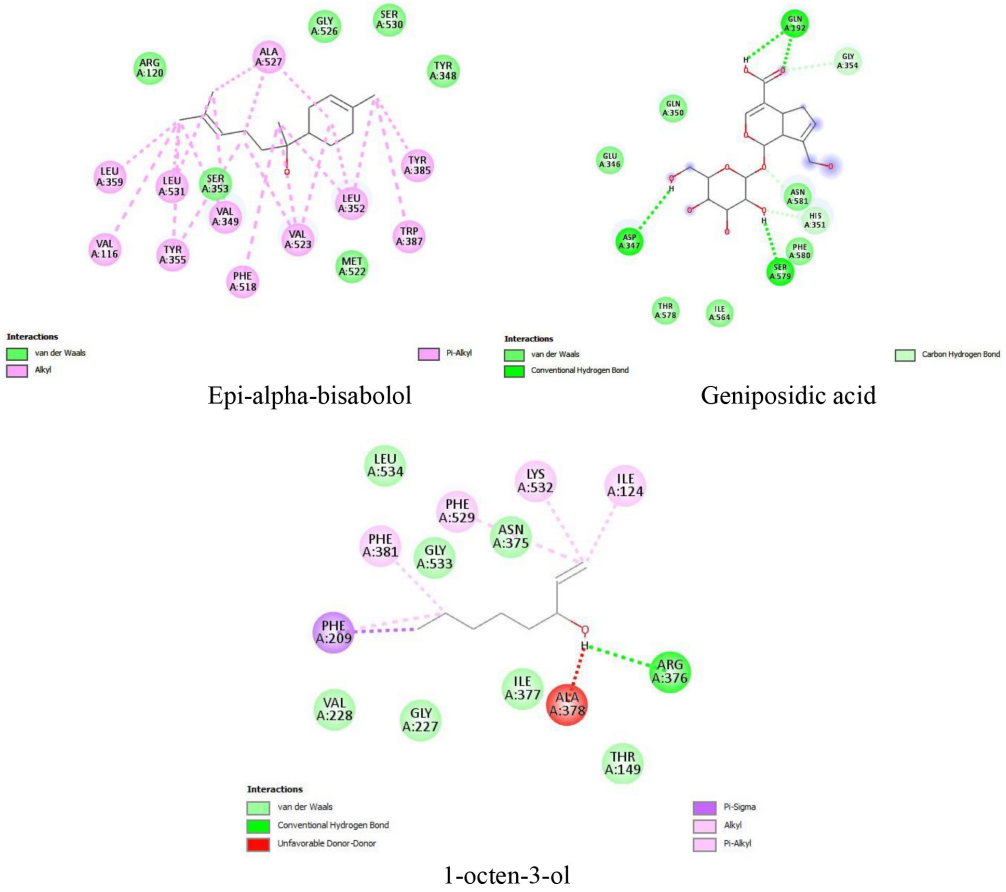


Figure 4. Molecular docking interactions data of Epi-alpha-bisabolol, Geniposidic acid, and 1-octen-3-ol with 6COX.

4. Conclusion

In this manuscript, the extraction of *P. barbata* was performed using chloroform and ethyl acetate, along with *in vitro* alpha amylase and protein denaturation inhibition assays of both the extracts, considering acarbose and aspirin as standard compounds. Chloroform extract of *P. barbata* showed good alpha amylase and protein denaturation inhibitory activities. As per *in silico* study data, geniposidic acid and epi-alpha-bisabolol may be responsible for antidiabetic and anti-inflammatory properties, respectively. These findings highlighted the antidiabetic and anti-inflammatory potentials of *P. barbata*.

5. Author Contribution

Formal Analysis and Procedure: A. D; Write and review: P.K.; Resources: V.K.; Supervision, Concept, Validation: S.S. All authors have read and agreed to the published version of the manuscript.

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