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Insilico Analysis of PHB from Halophiles for potential Bio-medical applications

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ABSTRACT

Polyhydroxybutyrate (PHB) is an important biopolymer accumulated by halophilic organisms. PHA is a family of polyesters is accumulated as granules in the cell of bacteria. Polyhydroxybutyrate (PHB) can be used as an alternative polymer to polylactide-glycolides for drug carrier production. It is a linear homopolymer biosynthesized by various strains of bacteria by condensation of D (-)-B-hydroxybutyric acid and used as an energy and carbon source. PHB can be obtained by extraction from bacteria or by chemical synthesis. To be suitable as drug carrier the PHB (polymer) has to be biocompatible, biodegradable in certain applications, and nontoxic. PHB seems to be biocompatible and biodegrades readily to carbon dioxide in bacteria; however, in humans, the reports are few and contradictory. The PHB was extracted from halophilic bacteria. The structural characterization of PHB was done by using NMR (nuclear magnetic resonance). To genenerate SMILES the structure were drawn in MarvinSketch. The PHB were screened based on the Lipinski's rule of 5. The PHB molecule was subjected to the toxicity analysis and those that passed the toxicity test analyzed for docking studies.
Keywords : PHB, Nuclear magnetic resonance (NMR), MarvinSketch, Insilico.

I. INTRODUCTION

Polyhydroxybutyrate (PHB) is an important biopolymer accumulated by halophilic organisms. PHA production by halophilic microorganisms has attracted much attention in recent years. PHBs are a type of PHA. Artificial polymers cause serious environmental issues because of their non-biodegradability. In order to reduce the amount of plastic waste, world-wide programs for efficient management of utilized plastic materials, such as recycling, have been started. Another solution to reduce plastic residues is the use of biodegradable plastics [1]. Polyhydroxyalkaonates (PHAs) are polyesters accumulated by different bacteria's under uneven growth conditions, when the carbon substrate is in excess and other nutrients such as nitrogen, sulfur, phosphorus or oxygen are in lacking [2]. Polyhydroxybutyrate (PHB) is intracellular carbon storage mechanisms for many species of halophilic microorganism. PHA is made by different microorganisms and can easily be broken down by them. Many studies have shown that PHA is readily degraded by a variety of microorganisms when placed in a natural ecosystem, such as the soil or aqueous environments [3].

PHA varities exhibit thermal and mechanical properties that are similar to petroleum-based plastics, such as polypropylene [4]. PHA materials must also not trigger immune responses during degradation in the body to be considered biocompatible. Typically,

PHA polymers are reducing by the action of non-specific lipases and esterase's in nature [5]. Bacterial PHAs could be divided into two groups depending on the number of carbon atoms in themonomeric units: short-chain-length (SCL) PHB, which consist of 3–5 carbon atoms, and medium-chain-length (MCL) PHB, which consist of 6–14 carbon atoms [6].The use of poly (3-hydroxybutyrate) (PHB) as carriers for drug delivery or scaffolds in tissue engineering.PHB have many advantages when compared to other chemically produced polymers like polyglycolate, polylactate, and poly(lactide-co-glycolide) which include excellent biocompatibility, biodegradability, easier processibility, and the controllable retarding properties which can be modulated by alternative in processing and molecular weight of the polymer composition. PHB in combination with other biocompatible and nontoxic polymers would also have an enhanced scope in biomedical applications [7].

II. METHODS AND MATERIAL

1. Insilico analysis of PHB

A) Thermal and mechanical properties:

Matweb tool predicts mechanical & thermal properties. Matweb tool supports for calculation of important properties Melting Temperature (Tm, °C), Tensile Strength (Mpa), Tensile modulus (GPa), Elongation at break (%) , Glass transition temperature (Tg, °C).

B) Virtual screening by Lipinski Rule of Five:

Molecular descriptors and drug likeliness properties of compounds were analyzed using the Molinspiration server with based on Lipinski's Rules. Molinspiration server supports for calculation of important molecular properties such as LogP, polar surface area, number of hydrogen bond donors and acceptors, as well as prediction of bioactivity score for the most important drug targets GPCR ligands, kinase inhibitors, ion channel modulators, enzymes and nuclear receptors (Lipinski CA *et al.*, 2001).

C) Virtual screening of ADMET properties analysis:

The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were predicted using admetSAR database. In admetSAR is a web based query tools incorporating a molecular build-in interface enable the database to be queried by SMILES and structural similarity search (S.K. Balani *et al.*, 2005).

2. Analysis of PHB for Biomedical application

A) Designing of standard ligands

The chemical structures of the selected ligands were drawn by using Chemsketch. Chemsketch is the powerful all-purpose chemical drawing and graphics package from ACD\Labs developed to help chemists quickly and easily draw molecules, reactions and schematic diagrams, calculate chemical properties, and design professional reports and presentations. ACD Chemsketch can convert SMILES notation to structure and vice versa. The drawn chemical structures were stored in PDB format.

B) Preparation of PHB & ligand conjugation

PHB Ligand conjugation prepared by using defined chemical structure of PHB & Ligand using Marvinsketch.

3. virtual screening of PHB tagged Ligand

A) Virtual screening by Lipinski Rule of Five:

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The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were predicted using admetSAR database. In admetSAR is a web based query tools incorporating a molecular build-in interface enable the database to be queried by SMILES and structural similarity search (S.K. Balani *et al.*, 2005).

C) Molecular docking of PHB tagged ligands and energy calculation

The molecular interaction and binding studies of designed chemical ligands with all the four proteins were carried out by molecular docking by using Hex software. The efficiency of ligand was determined by energy calculation tabulated for respective ligand and protein. Hex is an interactive Molecular Graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. Hex can also calculate Protein-Ligand docking, assuming the Ligand is rigid, and it can superpose pair of molecule using only knowledge of their 3D shapes. It uses Spherical Polar Fourier (SPF) correlation to accelerate the calculations and its one of the few docking programs which has built in graphics to view the result.

III. RESULTS AND DISCUSSION

1. Insilico analysis of PHB

A) Thermal and mechanical properties:

Matweb tool predicts mechanical & thermal properties. Matweb tool supports for calculation of important properties Melting Temperature (Tm, °C) , Tensile Strength (Mpa) , Tensile modulus (GPa) , Elongation at break (%) , Glass transition temperature (Tg, °C) as shown in Table 1 .

TABLE 1. Showing Mechanical and Thermal Properties of PHB

Properties	PHB
Melting Temperature (Tm, °C)	120
Tensile Strength (Mpa)	40.0Mpa
Tensile modulus (GPa)	3.50GPa
Elongation at break (%)	6
Glass transition temperature (Tg, °C)	4

B) Virtual screening by Lipinski Rule of Five:

Molecular descriptors and drug likeliness properties of compounds were analyzed using the Molinspiration server with based on Lipinski's Rules as shown in Table 2. Molecule which satisfy the Lipinski's rule i.e. log P ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5 and number of rotatable bond ≤ 10; were subjected for further analysis.

TABLE 2. Showing Predicted Important Molecular Properties

Compoud	LogP	MW	Non	Nohnh
[PHB]3	4.26	348.47	6	0
[PHB]4	5	464.63	8	0
[PHB]5	6.57	580.79	10	0

C) ADMET analysis:

The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of

the compounds were predicted using admetSAR database as shown in Table 3.

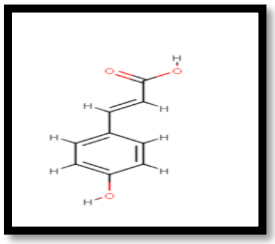
TABLE 3. Showing Predicted ADMET Properties

1. Analysis of PHB for Biomedical application

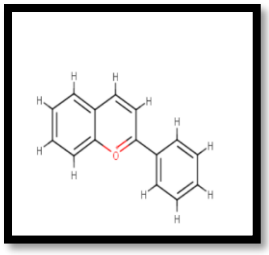
Compound	Brain blood barrier	Human intestinal absortion	Carcinogenicity	AMES toxicity
$[PHB]_3$	BBB+	HIA+	No Carcinogenicity	No AMES toxicity
$[PHB]_4$	BBB+	HIA+	No Carcinogenicity	No AMES toxicity
$[PHB]_5$	BBB+	HIA+	No Carcinogenicity	No AMES toxicity

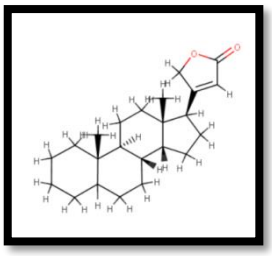
A) Designing of standard ligands

The chemical structures of the selected ligands were drawn by using Chemsketch. Based on molecular properties and toxicity properties of ligand structure were built and represented in figure 1 respectively.

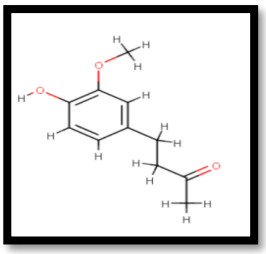


P-coumeric acid





Cardenolide

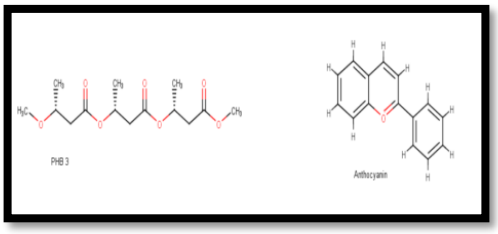


Zingerone

Figure1. Showing Structures of Standard Ligands

B) Preparation of PHB & ligand conjugation

PHB Ligand conjugation prepared by using defined chemical structure of PHB & Ligand using Marvinsketch. Conjugated PHB and Ligand structure represented in figure 2, 3, 4 and 5 respectively.



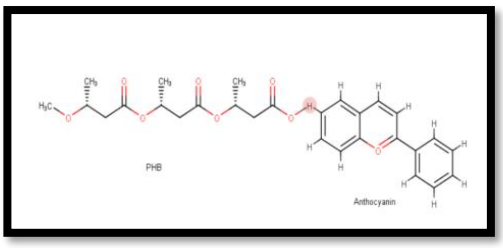
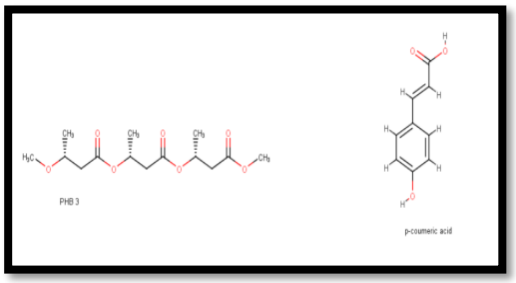


Figure 2. Showing Conjugated structure of PHB-Anthocyanin



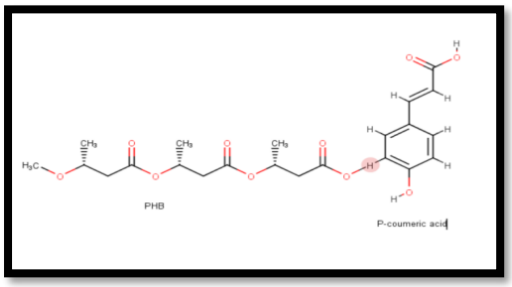
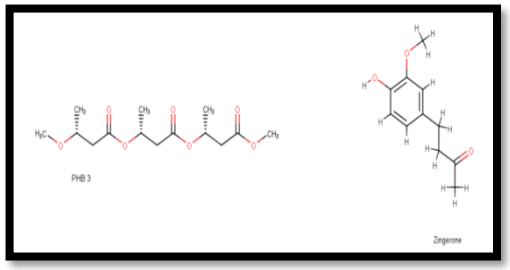


Figure 3. Showing Conjugated structure of PHB-P-coumeric acid



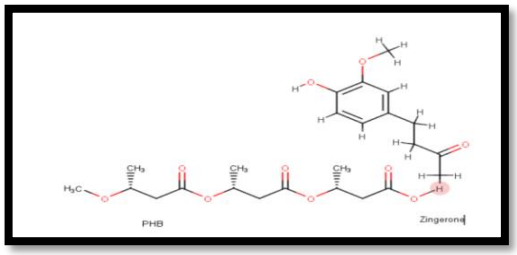
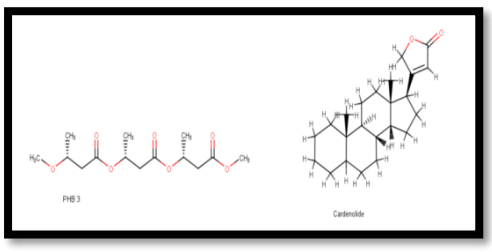


Figure 4. Showing Conjugated structure of PHB-Zingerone



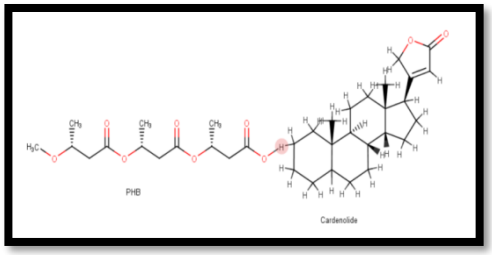


Figure 5. Showing Conjugated structure of PHB-Cardenolide

2. Virtual screening of PHB tagged Ligand

A) Virtual screening by Lipinski Rule of Five:

Molecular descriptors and drug likeliness properties of compounds were analyzed using the Molinspiration server with based on Lipinski's Rules as shown in Table 4. Molecule which satisfy the Lipinski's rule i.e. log P ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and number of hydrogen bond donors ≤ 5 and number of rotatable bond ≤ 10; were subjected for further analysis.

TABLE 4. Showing Predicted Important Molecular Properties

Compound	LogP	MW	nON	Nohnh
PHB-Anthocyanin	2.19	496.56	8	1
PHB-p-coumeric acid	2.53	453.46	10	3
PHB-Cardenolide	4.89	631.83	9	1
PHB-Zingerone	1.98	483.53	10	2

TABLE 5. Showing Predicted ADMET Properties

Compound	Brain blood barrier	Human intestinal absortion	Carcinogenicity	AMES toxicity
PHB-Anthocyanin	BBB+	HIA+	No carcinogenicity	No AMES toxicity
PHB-P-coumeric acid	BBB+	HIA+	No carcinogenicity	No AMES toxicity
PHB-Cardenolide	BBB+	HIA+	No carcinogenicity	No AMES toxicity
PHB-zingerone	BBB+	HIA+	No carcinogenicity	No AMES toxicity

B) Virtual screening of ADMET properties analysis:

The pharmacokinetic properties such as Absorption, Distribution, Metabolism, Excretion and Toxicity of the compounds were predicted using admetSAR database as shown in Table 5.

C) Molecular docking of PHB tagged ligands and Energy calculation

The molecular interaction and binding studies of designed chemical ligands with all the four proteins were carried out by molecular docking by using Hex software. The efficiency of binding of each ligand to individual protein was determined by calculating binding energy scores as represented in table 6. Docking between protein and ligand was shown in figure 6, 7, 8 and 9 respectively.

TABLE 6. Receptor–Ligand Binding Energy Scores

Ligand	Receptor	E total
PHB-Anthocyanin	Mammalian sterile 20-like Kinase-1(MST 1)	-328.95
PHB-p-coumeric acid	NS2BNS3 PROTEASE	-293.66
PHB-Cardenolide	NS2BNS3 PROTEASE	-358.92
PHB-Zingerone	X linked inhibitor of apoptosis(1c9q)	-250.83

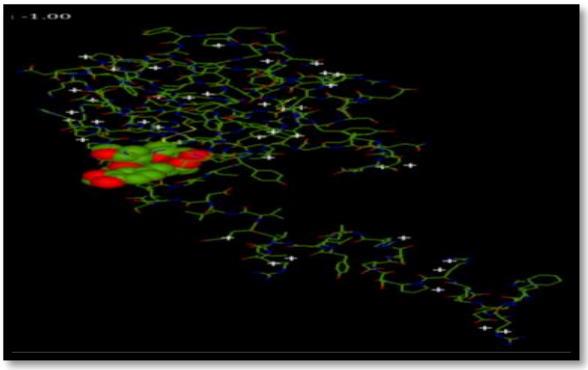


Figure 6. Showing receptor X linked inhibitor of apoptosis (1c9q) and Ligand (PHB-Zingerone) docking.

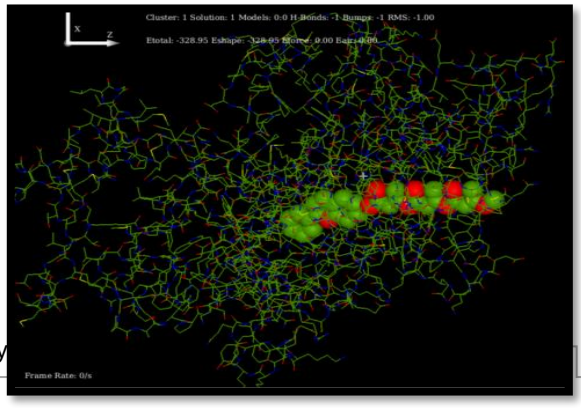


Figure7. Showing receptor Mammalian sterile 20-like Kinase-
1(MST1) and Ligand (PHB-Anthocyanin) docking.

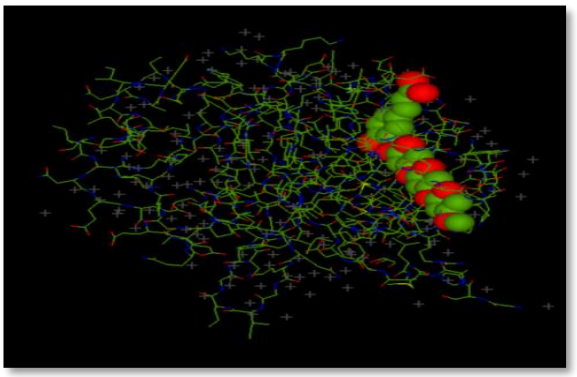


Figure8:Showing receptor NS2BNS3 PROTEASE and

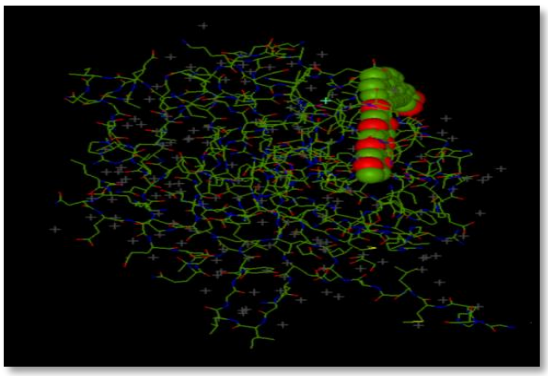


Figure9: Showing receptor NS2BNS3 PROTEASE and Ligand (PHB-Cardenolide) docking.

IV. CONCLUSION

In-silico screening of PHB to understand Thermal and Mechanical properties. The biochemical application of PHB as a drug delivery system was carried out by *in-silico* analysis of its molecular properties, Toxicity and metabolism, further the efficiency of PHB as a drug delivery component was assessed by tagging with standard drug and molecular docking with respective target proteins. Currently the PHB type's polymer has been employed for medical application such as sutures, implants, urological stents, neutral- and cardiovascular- tissue engineering, facture fixation, treatment of narcolepsy and alcohol addiction, drug delivery vehicles, cell microencapsulation, support of hypophyseal cell, or as precursor of molecules with anti-rheumatic, analgesics radiopotentiator, chemopreventive, antihelminthic or anti-tumoral properties. Increased interest in the use of PHB for medical applications had arisen the response to the need for the field of drug delivery, where a much wider range of biodegradable and biocompatible polymers are being use as drug carriers. Because of their versatility and wide range of properties, biodegradable PHBs are being used as novel drug delivery systems. PHB-based drug carrier holds tremendous promise in the areas of cancer therapy and controlled delivery of drugs including steroids, vaccines, and other biological molecules. They can be formulated for targeted drug delivery to tumours or organs.

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