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KEYWORDS: Shorea robusta, alpha amyrin, beta amyrin, H - Ras protein.

IN SILICO DOCKING AND ANTI-CANCER ACTIVITY OF THE ISOLATED COMPOUNDS (ALPHA AND BETA AMYRIN) FROM METHANOLIC BARK EXTRACT OF SHOREA ROBUSTA

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M. C. Kamaraj Centre for Product Development, Heavenly Fuel Pvt Ltd, Besant Nagar, Chennai - 600 090, Tamil Nadu, India

K. Olikkavi Department of Biochemistry & Biotechnology, Annamalai University, Annamalai nagar, Tamil Nadu, 608 002 India

L. Vennila Department of Biochemistry & Biotechnology, Annamalai University, Annamalai nagar, Tamil Nadu, 608 002 India

S.Subashchandra bose* Department of Physics, Centre for research and development, PRIST Deemed University, Thanjavur-613 403, India*Corresponding Author sscbphysics@gmail.com

S. Mohan Raj Department of Physics, Centre for research and development, PRIST Deemed University, Thanjavur-613 403, India

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ABSTRACT:

There is a constant demand to develop new, effective and affordable anti-cancer drugs. The traditional medicinal system is a valuable and alternative resource for identifying novel anti-cancer agents. The aim of the present study is to investigate the inhibitory activity of the compounds of methanolic bark extract of Shorea robusta on hepatocellular carcinoma by molecular docking studies on isolated compounds namely alpha amyrin and Beta amyrin. These compounds are used for docking on human oncogene protein 121p. Docking studies of designed compound were carried out using molecular docking server. The G recorded for alpha and beta amyrin binding with human Ras protein was -9.36 kcal/mol and -8.90 kcal/mol respectively. This insilico study demonstrates the interactions of active components of methanolic bark extract of Shorea robusta against hepatocellular carcinoma. Frontier singly occupied molecular orbitals (SOMO) were studied by Density functional theory (DFT) and Time dependent-DFT calculations.

1. Introduction

Cancer is an uncontrolled multiplication of cells within the body. It is a deadly disease which is characterized by a constant, abnormal and quite self-sufficient proliferation of cells, therefore the permanent cellular defect that is passed on to the progeny [1]. It is one of the most prominent diseases in humans and currently there is considerable scientific and feasible interest in the finding of new anticancer agents from natural sources. Hepatocellular carcinoma (HCC) is one of the most frequent cancers among humans, with 0.50–1 million newly diagnosed cases each year [2]. Carcinogenesis can occur as a result of chemical or biological damage to normal cells in a multistep process that involves changes at the beginning stage followed by promotion and progression which ultimately leads to malignancy. In developing countries about 35% of standard drugs are derived from natural products. Several investigations are being carried out worldwide to discover naturally occurring compounds which can hold back or prevent the progress of carcinogenesis [3]. In recent years, significant importance has been shown on the detection of plant products containing antioxidant properties as free radicals are considered to play a major role. However, molecular docking studies were not carried out in these plant compounds. Shorea robusta, a tropical hardwood originated and developed in the South-East Asia. It grows most commonly in Indonesia but can also be seen in Malaysia, the Philippines and certain parts of the Southern India [4]. Traditionally the plant is used for the treatment of dysentery, ulcers, jaundice, wounds, gonorrhea, and leprosy. The major chemical constituents of S. robusta are reported to contain flavonoids, steroids, terpenoids, phenols and cardioglycosides [5]. The development of tumors is accompanied by characteristic alterations in the activities of enzymes, mainly those involved in carbohydrate metabolism. In vitro and in vivo studies of anticancer activity is time consuming and has extensive, expensive and elaborative lab work studies, whereas in insilico approaches, it is possible to find out the interaction between target protein and ligand molecule in short duration using docking server tools. Hence in the present study, an attempt was made to evaluate the anticancer activity of the chosen plant compound namely alpha and beta amyrin through in Silico approaches.

2. Materials and methods

2.1 Molecular Docking

A computational tool offers the advantage of delivering new drug candidates more quickly and at a lower cost. The present work by computational approach used for the following software manipulation of drugs using molecular docking server online web service for calculation of drug likeness. The identified compounds from methanolic bark extract of Shorea robusta was used to interact with human oncogene proteins (H-Ras protein) retrieved from PDB.

2.2 Protein Data Bank (PDB)

The PDB is the particular, international database for information about the 3D structure of biomacromolecules and their complexes as determined by X-ray crystallography, NMR spectroscopy and includes more than a few Nobel Prize winning structure. Human oncogene protein was downloaded from protein data bank with the specific resolution and the PDB id is 121p.

2.3 Protein sequences

The human oncogen protein was retrieved from the online database of SWISSPROT [6]. It was obtained through the entry keyword of oncogene protein HRSA and searched the entire database. Different sequences of oncogene protein were shown. Among that the human oncogene protein was retrieved in FASTA

format and it was used for the further computational analysis.

2.4 Primary structure prediction
For physiochemical characterization, theoretical isoelectric point (PI), molecular weight, total number of positive and negative residues, extinction coefficient [7], Half-life [8, 9, 10, 11], instability index [12], aliphatic index and grand average of hydropathy (GRAVY) [13] were computed using the Expasy protparm server.

2.5 Secondary structure prediction
Secondary structure of the protein was determined by using the FASTA sequences of protease and predicted using SOPMA and SOPM [14].

2.6 Transmembrane region identification
The transmembrane region of oncogene protein was examined by SOSUI server [15]. The evaluated transmembrane region was analysed and visualized by Pepwheel [16] using EMBOSS 2.7 suit.

2.7 Homology modeling and validation
The protein sequence was subjected for comparative homology modeling via Swiss model [17] and evaluate by Rampage online server [18]. The protein was confirmed by using online server Prochek [19] and WHAT IF [20]. The Swiss model executes the sequence alignments and looks for the assumed template protein in the 3D model.

2.8 Sequence subjected for modeling:
>target
MTEYKLVVVGAGGVGKSALTIQLIQNHFVDEYDPTIEDSYRKQVVIDGET
CLLDILDTAGQEEYSAMRDQYMRTGEGFLCVFAINNTKSFEDIHQYREQI
KRVKDSDDVPMVLVGNKCDLAARTVESRQAQDLARSYGIPYIETSAKTR
QGVEDAFYTLVREIRQHKLRKLNPPDESGPGCMSCKCVLS

2.9GC-MS analysis
Extract of Shorea robusta was subjected to GC-MS analysis and the distinguished compounds were identified.

2.10 Ligand retrieved
The screened compounds were retrieved from the pubchem compound (http://www.ncbi.nlm.nih.gov/pccompound) and used for the further studies.

2.11 Docking studies
Docking calculations were carried out using Docking Server [21]. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on alpha amyrin and beta amyrin.pdb protein model. Essential hydrogen atoms, Kollman united atom type charges, and salvation parameters were added with the aid of Auto Dock tools. Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program [22]. AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method [23]. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 10 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

3. Computational details
The quantum chemical calculations of phytochemical structure of methanolic bark extract of Shorea robusta have been performed using the B3LYP level of theory supplemented with the standard 6-311 G(d,p) basis set, using the Gaussian 03 program. The entire calculations were performed at DFT levels on a personal computer using Gaussian 03W [24] program package, invoking gradient geometry optimization [25].

4. Results and Discussion
4.1 Primary structure analysis
The human oncogene protein was retrieved from SWISSPROT in FASTA format. The primary physiochemical parameter was performed and amino acid composition was identified (Table 1 and Table 2). The result shows that the oncogene protein was composed of 22 aminoacids with different ratios. Among that valline content was more (8.5%) that indicate the hydrophobic nature of protein because it has an aliphatic isobutyl side chain and also essential aminoacid. This indicates the sequence length is 189, and the molecular weight of protein was found to be 21298.13, the protein has 5.16 isoelectric points that represent the protein is basic in nature and it will help to purify the protein molecule. The number of negative charged residues (Asp + Glu) is 29 and number of positive charged residues (Arg + Lys) is 23.The extinction coefficient was 13785 at 280 nm; it may be probable to avoid interference of other substances. The evaluated value used to determine the quantification of protein – protein or protein – ligand interactions. The quantitative measurement of dynamic equilibrium based on the half-life time. The H-RAS P21protein has 30 hours in mammalian reticulocytes; in yeast have 20 hours and 10 hours in E.coli. The stability of protein was determined by using the instability index (43.27). The aliphatic index characterize that the volume of protein occupied by aliphatic chains (Alanine, Valine, Isoleucine and Leucine), oncogene protein have 81.96 that denoted unstable in high thermal conditions. Grand Average Hydropathicity denoted that the hydrophobicity of aminoacid residues. Here oncogene protein has -0.417 had a reasonable interaction with water molecule. The protein molecule has 4 different atoms such as C, H, N, O and S, molecular formula was C926H1478N258O295S11.

4.2 Secondary structure of protein
The secondary structure of H-Ras protein was predicted by using SOPMA and SOPM Table 3. The protein was α helix with other structures such as extended stand, β turn and random coil. Presents the comparative analysis of SOPMA and SOPM. From which it is clear that random coil is mostly present, when the structure was predicted both by SOPMA and SOPM, followed by extended strand and alpha helix. So this protein is stable in nature.

4.3 Protein structure validation
4.3.1 Ramachandran plot
The predicted human oncogene protein structure was validated by using Ramachandran plot using PROCHECK software that shows the protein molecule contains 166 residues in that 138 aminoacid most favored region, 12 aminoacid additionally allowed and 0 aminoacid generally and disallowed region. The results are shown in Fig 1. WHAT IF shows that i.e. Z-score of protein is -1.69.

4.3.2 Docking
Human oncogen protein Fig 2, and ligands (isolated compounds of Shorea robusta) Table 4 were subjected to docking studies by using online Auto dock server. The software used to runs 10 docking and were shown in Table 5. The 3D structure of human oncogene protein (PDB id: 121p) were optimized to achieve minimal potential energy using molecular docking server. The minimization values are summarized. Docking simulation of 10 runs of plant compound alpha and beta amyrin was performed for a set of catalytic active site of human oncogene protein (Fig 3 and Fig 4). The best docked conformation was selected based on lowest docking energy and binding free energy. Docking score is a measure of interaction of the ligand to the active site of the target [26]. More negative values indicate more effective stable conformation of the bound ligand-target. 121P is the human oncogene protein known as Ras protein, which bind GDP/GTP and possess intrinsic GTPase activity. These enzyme alternates between an inactive form bound to GDP and an active form bound to GTP. It is activated by a Guanine Nucleotide-Exchange Factor (GEF) and inactivated by a GTPase-activating

protein (GAP).

Docking simulation of alpha amyrin into H-Ras protein (PDB id: 121p) resulted in the formation of amino acid residues phe 28, Val 29 are involved in hydrophobic interactions. Asp 30 is the polar bond interaction, and the other interactions are Thr 35, Lys 117, Ser 17. The docking score of the complex were -9.36 kcal/mol. The docking result of Beta amyrin with human oncogene protein (PDB id: 121p) showed that the amino acid residues are Val 29, Leu 120, Phe 28, Ala 18 are involved in hydrophobic interactions with an active site and Lys 117, Asp 30, Lys 147, Asp 119 are involved in other interactions. The docking score of the complex was found to be -8.90 kcal/mol.

4.4 Band gap energy analysis using TD-DFT calculation

The frontier molecular orbital energies, the shapes and symmetries of the HOMO and LUMO are crucial in predicting the reactivity of a group and the stereo chemical and region chemical outcome of a chemical reaction [27]. The higher HOMO value shows that the molecule has good electron donating ability; as well as the lower value implies weak electron donating ability. A smaller energy gap (between the LUMO and HOMO) of the hit molecules illustrates that they are high reactive [28]. Consequently, the highest occupied state reveals that the readily available electronic transfer between the frontier MOs. In this molecule, singly occupied MOs appeared, in which 88th MO (βHOMO) has singly occupied, due to unpaired electron in the carbon ring, whereas αHOMO filled up to 87th MO, the singly occupied 87th MO is due to an Oxygen unpaired atom. In alpha amyrin compound, the energy of LUMO is same for both α and β unoccupied states (Fig. 5). On the other hand, in beta amyrin compound, 118 MOs are singly occupied as α-MO, 119 MOs are occupied as β-MO and it is being singly occupied HOMO state (Fig. 6). Similarly, α and β LUMO are 119 and 120 MO respectively, which are having different energies. Among the calculated band gap energies β-MOs of Beta amyrin compound has very narrow band gap (-1.057eV), from this investigation it clearly seems that the Beta Amyrin has more biological activity than the Alpha amyrin compound. The energies of the MOs are listed in Table S1 (Supporting information).

5. Conclusion

The human oncogene protein plays a vital role in anti cancer activity, a detailed study of the physiochemical characteristics helps to understand its role in anti-cancer activity. The physiochemical parameters also supported the protein properties, and then through pocket finder, the active sites in the protein that were suitable for binding with ligand in docking studies. On the other hand, Shorea robusta containing organic compounds was made to interact with the human oncogen H-Ras protein and this provides better scoring through Autodock. Among the calculated band gap energies, the β-MOs of Beta amyrin compound has very narrow band gap (-1.057eV), from this investigation it clearly seems that the Beta Amyrin has more biological activity than the Alpha amyrin compound. These findings enlighten the anticancer activities of alpha and beta amyrin in Shorea robusta and which needs further biochemical findings to confirm it.

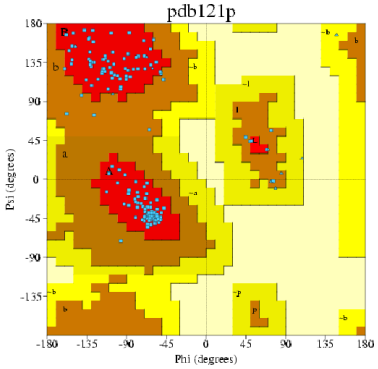


Fig: 1. Ramachandran plot

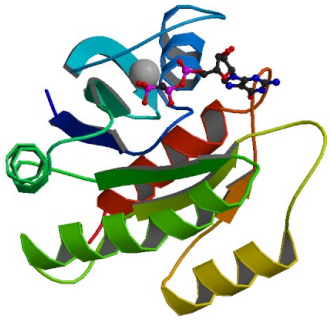


Fig.2. H-Ras Protein

Docking results

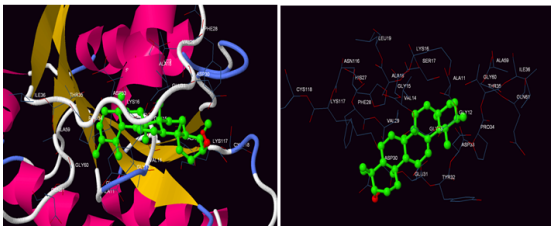


Fig.3. alpha amyrin with H-Ras protein (121p)

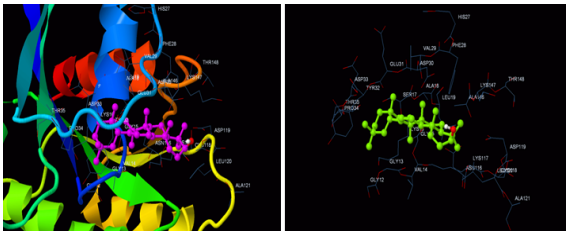


Fig.4. Beta amyrin with H-Ras protein (121p)

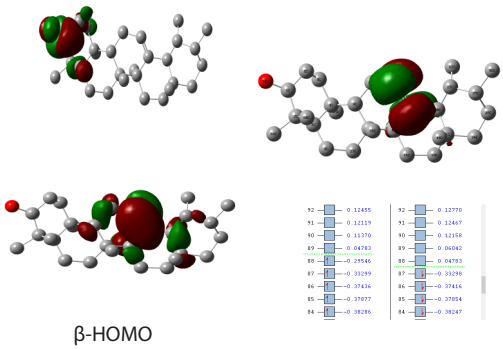


Fig 5. Plots of HOMO and LUMO alpha amyrin

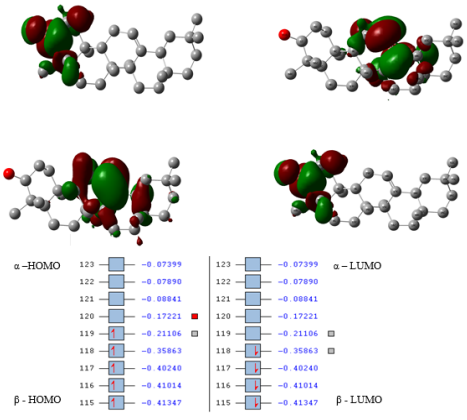


Fig 6 Plots of HOMO and LUMO beta amyrin

List of Tables

Table: 1. Amino acid composition (%) of stable factor computed in protparam

Amino acids	Numbers	Percentage
Ala (A)	11	5.8%
Arg (R)	12	6.3%
Asn (N)	5	2.6%
Asp (D)	15	7.9%
Cys (C)	6	3.2%
Gln (Q)	11	5.8%
Glu (E)	14	7.4%
Gly (G)	13	6.9%
His (H)	3	1.6%
Ile (I)	11	5.8%
Lea (L)	14	7.4%
Lys (K)	11	5.8%
Met (M)	5	2.6%
Phe (F)	5	2.6%
Pro (P)	6	3.2%
Ser (S)	11	5.8%
Thr (T)	11	5.8%
Trp (W)	0	0.0%
Tyr (Y)	9	4.8%
Val (V)	16	8.5%
Pyl (O)	0	0.0%
Sec (U)	0	0.0%

Table: 2. Parameters computed using Expasy's protparam tool.

Name	Accession number	Sequence length	Mol. Wt	PI	-R	+R	EC	II	AI	GRAVY
HRAS	Po1112	189	21298.1	5.16	29	23	13785	43.27	81.96	-0.417

Mol.wt- Molecular Weight; PI- Isoelectric point; -R – number of negatively charged residues; +R – number of positively charged residues; EC – Extinction coefficient at 280nm; II – Instability Index; AI – Aliphatic Index; GRAVY – Grand average of Hydropathicity.

Table: 3. Secondary structure of H-Ras protein by SOPMA and SOPM

Secondary structure	SOPMA	SOPM
Alpha helix (Hh)	85 is 44.97%	85 is 44.97%
310 helix (Gg)	0 is 0.00%	0 is 0.00%
Pi helix (Ii)	0 is 0.00%	0 is 0.00%
Beta bridge (Bb)	0 is 0.00%	0 is 0.00%
Extended strand (Ee)	33 is 17.46%	33 is 17.46%
Beta turn (Tt)	19 is 10.05%	19 is 10.05%
Bend region (Ss)	0 is 0.00%	0 is 0.00%
Random coil (Cc)	52 is 27.51%	52 is 27.51%
Ambiguous states (?)	0 is 0.00%	0 is 0.00%
Other states	0 is 0.00%	0 is 0.00%

Table .4. Plant compounds identified by GC-MS

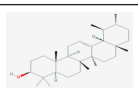
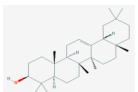
S. No	Name of the compound	Mol. Formula	Mol. Weight	Mol. structure
1	Alpha amyrin	C30H50O	426.729	
2	Beta amyrin	C30H50O	426.729	

Table: 5. Interacting residues responsible for docking:

Docking result	Est. free energy of binding	Est. inhibition constant, Ki	vdW + Hbond + desolv Energy	Electrostatic Energy	Total Interm olecs. Energy	Frequency	Interact. Surface
Alpha amyrin with 121p	-9.36 kcal/mol	136.68 nM	-9.61 kcal/mol	-0.05 kcal/mol	-9.66 kcal/mol	100%	902.319
Beta amyrin with 121p	-8.90 kcal/mol	297.80 nM	-9.21 kcal/mol	+0.01 kcal/mol	-9.20 kcal/mol	100%	909.745

Table S1. The frontier molecular orbital energies of Alpha and Beta Amyrin

	α-HOMO eV	β-HOMO eV	α-LUMO eV	β-LUMO eV	Energy gap (ΔE) eV
Alpha amyrin	-9.061	-8.040	1.302	1.302	-7.759eV (α), -6.738 eV (β)
Beta amyrin	-9.759	-5.743	-5.758	-4.686	-4.001 eV (α), -1.057 eV (β)

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