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An *in vitro* studies Bromelain Enzyme used for Inhibition of Leukemia

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Abstract:
The modern-day have a study cited the anti-cancer interest of Bromelain closer to receptor for superior glycationend merchandise (RAGE) have become analyzed the use of in silico assessment. Insilico analysis protected structural assessment of RAGE and Bromelain, their disorder prediction and discovered through power optimization and docking.

Keywords: Leukemia; Bromelain Enzyme; Vitro

I. Introduction

Pharmacological marketers with modulation of anti-inflammatory, proteolytic, platelet aggregation inhibition and prostaglandin synthesis have been taken into consideration to be useful in regulating tumor boom and its metastasis. Bromelain, with comparable regulating actions, has proven protecting residences on tumor cell boom retardation and lung metastasis. The proof for the anti-most cancers hobby of Bromelain comes from traditional observations (in Southeast Asia), studies of animal- and cellular-based models and anecdotal scientific research. The anti-cancer hobby of Bromelain is attributed predominantly to its protease components(Maurer, 2001). A recent have a look at on human cancer cells showed that Bromelain should act at once to kill cancer cellsand was possibly extra than only a aid for different most cancers therapies. It immediately knocked out the middle aberrant gene signal, NF-Kappa B, together with the irritation that is normal in most cancers. however, in the most cancers cells themselves, it unleashed big loose radical harm that triggered the most cancers cells to die (something it does not do to wholesome cells). some other current study in human breast most cancers cells showed that Bromelain could spark off the method of autophagy in cancer cells, causing them to “eat themselves” and die. Autophagy is a normal residence cleansing technique in healthful cells. In most cancers cells the manner is hijacked. The fact that Bromelain can turn the tables on cancer cells is pretty exciting. Enzymes consisting of Bromelain are critical to break down proteins used to drive metabolic functions in the frame. Processed and overcooked ingredients were stripped of the herbal enzymes that in the beginning existed, and the pancreas is forced to paintings past its capacity to interrupt down digested proteins. most cancers cells also use a protein protect to cloak themselves and keep away from detection from the immune system. Protease enzymes help spoil the protein bond round cancer clusters so the body can break developing tumor wall systems and thwart cancer initiation. there's gathering evidence displaying the position of NF-kappa B signaling and over-expression in many styles of cancers (Ferrisand Grandis, 2007). rising proof additionally shows that depending on mobile context, NF-kappa B also can sell tumor suppression (Chen et al., 2008). among more than one target genes of NF-kappa Bis Cox-2, a key participant in continual and cancer-related infection (Hussain and Harris, 2007). Cox-2 is involved within the synthesis of prostaglandin E2 (PGE2), a pro-inflammatory lipid that still acts as an immunosuppressant and promoter of cancer development. by facilitating conversion of arachidonic acid into PGE2, Cox-2 was shown to promote tumor angiogenesis and development. it's far taken into consideration that inhibiting NF-KappaB, Cox-2 and PGE2 hobby has ability as a remedy of most cancers and chronic inflammatory diseases. Bromelain changed into proven to down-alter NF-KappaB and Cox-2 expression in mouse papillomas (Kalra et al., 2008) and in models of pores and skin tumorigenesis (Bhui et al., 2009). moreover, in human monocytic leukemia and murine microglial cellular strains, Bromelain was proven to inhibit bacterial endotoxin (LPS)-triggered NF-KappaB interest in addition to the expression of PGE2 and Cox-2 (Huang et al., 2008). The science now supports using Bromelain as part of a herbal assist strategy for any most cancers treatment or for prevention. As extra research is achieved the suitable mechanisms of Bromelain in the battle on cancer may be higher understood. The facts to this point shows that Bromelain is but some other nutrient, like green tea, curcumin, quercetin, resveratrol and tocotrienols that are able to inform the distinction among cancer cells and healthful cells, helping to kill the former at the same time as helping the survival of the wholesome cells.

II. Materials And Methods

A. Bromelain Anticancer Activity Through In Silico Analysis

1. Structural Analysis Primary Structure Prediction:

Expasy Prot Param Tool: ProtParam: is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBLor for a user entered sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

Secondary Structure Prediction Method: SOPMA is a secondary structure prediction method. SOPMA (Self-Optimized Prediction Method with Alignment) is an improvement of SOPM method. These methods are based on the homologue method of Levin et al. SOPMA correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins. Joint prediction with SOPMA and a neural networks method (PHD) correctly predicts 82.2% of residues for 74% of co-predicted amino acids.

Tertiary Structure Prediction:

CPH model: Cphmodels-3.0 is a web-server predicting protein 3d-structure by use of single template homology modeling. The server employs a hybrid of the scoring functions of cphmodels-2.0 and a novel remote homology-modeling algorithm. A query sequence is first attempted modeled using the fast cphmodels-2.0 profile-profile scoring function suitable for close homology modeling. Cph models-3.0 method is a high performing 3d-prediction tool. Beside its accuracy, one of the important features of the method is its speed. For most queries, the response time of the server is less than 20 minutes.

2. Disorder Prediction Method:

RONN (Regional Order Neural Network): The Regional Order Neural Network (RONN) server was developed as collaboration

between the OPPF and Exeter University as a tool for detecting regions of a sequence which are likely to be disordered. Apart from helping to understand potential functional aspects of a protein sequence, this information is also very valuable in construct design since long disordered sequences can adversely affect protein solubility, crystallizability and diffraction quality. In many cases it may even prevent successful structure determination altogether. The RONN server takes a sequence in FASTA format and produces a plot of the predicted probability of disorder for each residue in the sequence. Residues with a disorder probability >50% are predicted to be disordered. The method involves non-gapped sequence alignment between windows along the query sequence and a set of prototype sequences known to be disordered. A neural network is then used to assign a probability of disorder based on the quality of these alignments. RONN has proven itself to be one of the best methods for disorder prediction and is particularly suited to predictions for partially disordered sequences.

Argus Lab: Argus Lab is a molecular modeling program that runs on Windows 98, NT, and 2000. Argus Lab consists of a user interface that supports OpenGL graphics display of molecule structures and runs quantum mechanical calculations using the Argus compute server. The Argus compute server is constructed using the Microsoft Component Object Model (COM). Argus Lab is a program to build graphic representations of molecular models. Using this program, you will be able to show molecular models to pupils, or even design matters by combining different elements. You will be able to include in your model several atoms, residues, groups and calculations. Every component can be edited to meet your needs. You can use hydrogen, carbon, nitrogen, oxygen, chlorine and fluorine atoms. You can join those atoms using any kind of bond possible. This way you will be able to build simple or complex molecules.

SYBYL: SYBYL is a comprehensive computational tool kit for molecular design and analysis, with a special focus on the creation of new chemical entities. SYBYL provides essential construction and analysis tools for both organic and inorganic molecular structures, and much, much more. SYBYL/Base gives you access to building tools, molecular mechanics, quantum mechanical calculations, molecular dynamics, docking, geometric measurements, molecular comparisons (fits), surfaces and grid displays, journaling, annotation, hard copy, a programming language, an object manager, and the Molecular Spreadsheet; all are included in the core module. Large and small molecules are modeled in the same window, with no special tricks or separate setups.

Hex Protein Docking: Hex is an interactive protein docking and molecular superposition program. It takes protein and DNA PDB file format and also read small-molecule SDF files.Hex will run on most Windows-XP**,** Linux and Mac OS X PCs. On a modern PC, docking times range from a few minutes when the search is constrained to known binding sites, to about half an hour for a blind global search **(**or just a few seconds with CUDA**)**. On multi-processor Linux systems, docking calculation times can be reduced in almost direct proportion to the number of CPUs and GPUs used.

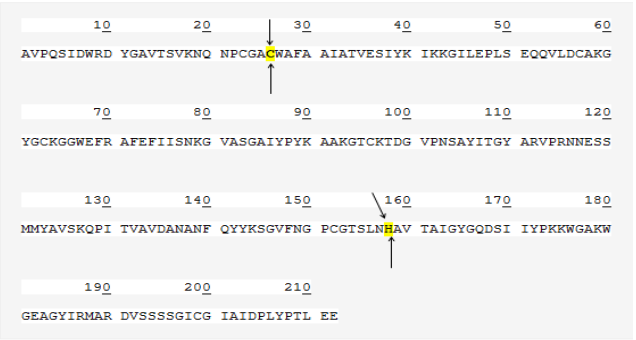
III. RESULTS

A. Bromelain Anti-Cancer Properties Through Insilico Analysis

1. Structural analysis of Bromelainenzyme:

Primary structure prediction: (ExpasyProtparam Tool) Bromelain (EC=3.4.22.32):

C and H are the active sites



Number of amino acids: 212
Molecular weight: 22830.9
Theoretical pI: 8.60
Amino acid composition:
Ala (A) 25 11.8%
Arg (R) 6 2.8%
Asn (N) 10 4.7%
Asp (D) 8 3.8%
Cys (C) 7 3.3%
Gln (Q) 7 3.3%
Glu (E) 9 4.2%
Gly (G) 22 10.4%
His (H) 1 0.5%
Ile (I) 17 8.0%
Leu (L) 6 2.8%
Lys (K) 15 7.1%
Met (M) 3 1.4%
Phe (F) 6 2.8%
Pro (P) 11 5.2%
Ser (S) 17 8.0%
Thr (T) 9 4.2%
Trp (W) 5 2.4%
Tyr (Y) 14 6.6%
Val (V) 14 6.6%
Pyl (O) 0 0.0%
Sec (U) 0 0.0%
(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 17
Total number of positively charged residues (Arg + Lys): 21
Atomic composition: Carbon C 1024 Hydrogen H 1567 Nitrogen N 269 Oxygen O 304 Sulfur S 10
Formula: C1024H1567N269O304S10
Total number of atoms: 3174
Extinction coefficients: Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water. Ext. coefficient 48735 Abs 0.1% (=1 g/l) 2.135, assuming all pairs of Cys residues form cystines. Ext. coefficient 48360 Abs 0.1% (=1 g/l) 2.118, assuming all Cys residues are reduced.
Estimated Half-Life: The N-terminal of the sequence considered is A (Ala). The estimated half-life is: 4.4 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).

Instability index:
The instability index (II) is computed to be 26.22 This classifies the protein as stable.
Aliphatic index: 73.25
Grand average of hydropathicity (GRAVY): -0.158 **2.**
Secondary Structure Prediction Method (SOPMA):
SOPMA result for : UNK_167830 (Bromelian)

```
        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
AVPQSIDWRDYGAVTSVKNQNPCGACWAFAAIATVESIYKIKKGILEPLSEQQVLDCAKGYGCKGGWEFR
ccccccccctttceeeccccccccchheehhhhhhhhhhhhhcttceeeeechhhhhhhhhccccccccchhh
AFEFIISNKGVASGAIYPYKAAKGTCKTDGVPNSAYITGYARVPRNNESSMMYAVSKQPITVAVDANANF
hhheeettccccccccccccccccccccccccceeeeetccccccccchhhhhhhhhtccceeeeecccce
QYYKSGVFNGPCGTSLNHAVTAIGYGQDSIIYPKKWGAKWGEAGYIRMARDVSSSSGICGIAIDPLYPTL
eeecccceeccccccccteeeeeeeccctteeeecccccccccttceeeeecccccccccceeeeccccccch
EEhh
```

Sequence length : 212

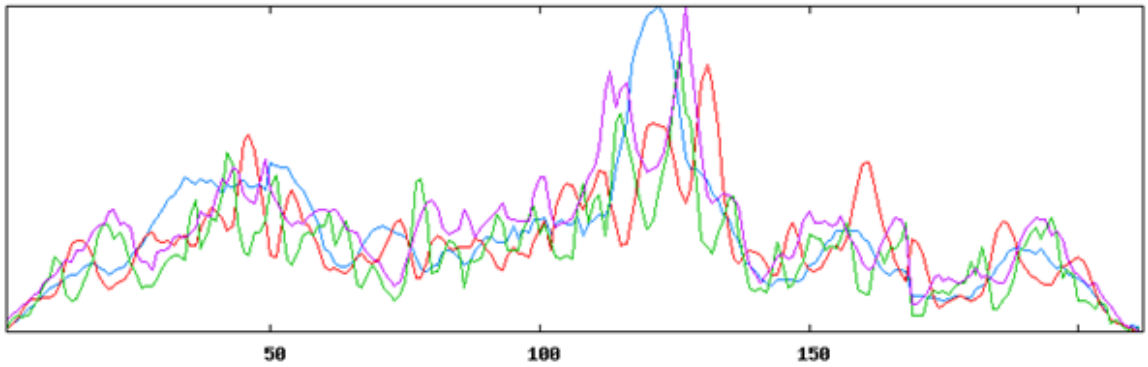


Fig.1.Secondary structure prediction of Bromelain enzyme.

SOPMA: Alpha helix (Hh) : 41 is 19.34%
310 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 48 is 22.64%
Beta turn (Tt) : 14 is 6.60%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 109 is 51.42%
Ambigous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

2. Tertiary Structure Prediction: CPH Model: From the above tertiary structure analysis the best PDB id selected was **1PCI_A** based on its maximum score and identity.

B. Structural Analysis Of (RAGE) Receptor Primary structure prediction: (Expasy Protparam Tool):
Number of amino acids: 419
Molecular weight: 48013.7
Theoretical pI: 9.64
Amino acid composition:
Ala (A) 20 4.8%
Arg (R) 29 6.9%
Asn (N) 14 3.3%
Asp (D) 17 4.1%
Cys (C) 10 2.4%
Gln (Q) 25 6.0%
Glu (E) 23 5.5%
Gly (G) 25 6.0%
His (H) 12 2.9%
Ile (I) 24 5.7%
Leu (L) 43 10.3%
Lys (K) 37 8.8%
Met (M) 13 3.1%
Phe (F) 14 3.3%
Pro (P) 29 6.9%
Ser (S) 31 7.4%
Thr (T) 14 3.3%
Trp (W) 2 0.5%
Tyr (Y) 18 4.3%
Val (V) 19 4.5%
Pyl (O) 0 0.0%
Sec (U) 0 0.0%
(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%
Total number of negatively charged residues (Asp + Glu): 40
Total number of positively charged residues (Arg + Lys): 66
Atomic Composition:
Carbon C 2138
Hydrogen H 3422
Nitrogen N 608
Oxygen O 602
Sulfur S 23
Formula: C2138H3422N608O602S23
Total number of atoms: 6793
Extinction coefficients: Extinction coefficients are in units of M-1 cm-1, at 280 nm measured in water. Ext. coefficient 38445 Abs 0.1% (=1 g/l) 0.801, assuming all pairs of Cys residues form cystines. Ext. coefficient 37820 Abs 0.1% (=1 g/l) 0.788, assuming all Cys residues are reduced.
Estimated half-life: The N-terminal of the sequence considered is M (Met). The estimated half-life is: 30 hours (mammalian reticulocytes, in vitro). >20 hours (yeast, in vivo). >10 hours (Escherichia coli, in vivo).
Instability index: he instability index (II) is computed to be 64.51 This classifies the protein as unstable.
Aliphatic index: 80.29
Grand average of hydropathicity (GRAVY): -0.548
Secondary Structure Prediction Method (SOPMA) SOPMA Result For : Q9UQ07

```
         10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MKNYKAIGKIGEGTFSEVMKMQSLRDGNYYACKQMKQRFESIEQVNNLREIQALRRLNPHPNILMLHEVV
hhheeeeehcttcchheeehhhcctttheeehhhhhhhhhhhhhhhhhhhhhhhhcccccceeeehhee
FDRKSGSLALICELMDMNIYELIRGRRYPLSEKKIMHYMYQLCKSLDHIHRNGIFHRDVKPENILIKQDV
ectttcchheeehhhhhhhhhhhhhttccccchhhhhhhhhhhhhhhhhhhhtttteecccccttceeeettc
LKLGDFGSCRSVYSKQPYTEYISTRWYRAPECLLTDGFYTYKMDLWSAGCVFYEIASLQPLFPGVNELDQ
cceehttccccccccccccceeeeeeeccchheecccccccccehhhhhhhhhhhhtcccccccccchhh
ISKIHDVIGTPAQKILTKFKQSRAMNFDFPFKKGSGIPLLTTNLSPQCLSLLHAMVAYDPDERIAAHQAL
hhhhhhetcccchhhhhhhhccccccccccccccccchhhccccchhhhhhhhhhhecctttcccchhhhh
QHPYFQEQRKTEKRALGSHRKAGFPEHPVAPEPLSNSCQISKEGRKQKQSLKQEEDRPKRRGPAYVMELP
hhhhhhhhhhhhhhhcccccccccccccccccccccccccccccchhccccccccccccccccccccccc
KLKLSGVVRLSSYSSPTLQSVLGSGTNGRVPVLRPLKCIPASKKTDPQKDLKPAPQQCRLPTIVRKGGR
ccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccccceeecccct
```

Sequence length : 419

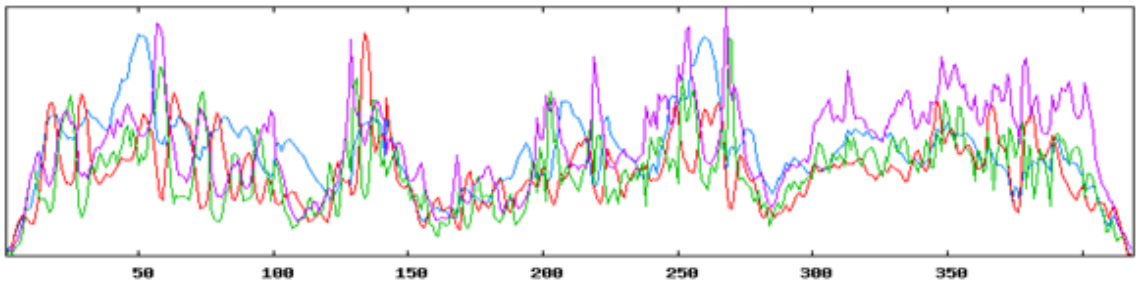


Fig.2.Secondary structure prediction of RAGE.

SOPMA:
Alpha helix (Hh) : 138 is 32.94%
310 helix (Gg) : 0 is 0.00%
Pi helix (Ii) : 0 is 0.00%
Beta bridge (Bb) : 0 is 0.00%
Extended strand (Ee) : 44 is 10.50%
Beta turn (Tt) : 24 is 5.73%
Bend region (Ss) : 0 is 0.00%
Random coil (Cc) : 213 is 50.84%
Ambigous states (?) : 0 is 0.00%
Other states : 0 is 0.00%

Parameters:
Window width:17,
Similarity threshold :8,
Number of states:4

Coils constitute most of the secondary structures, followed by alpha helix.

Tertiary Structure Prediction Hhpred: Common PDB ID is searched from the results of the above 3 tools. IF common PDB ID is matched and found then its structure can be used.Common PDB ID was not found from my results. Based upon the sequence identity and gap percentage, ID should be selected as the best one. I have selected3TTJfrom 100 HHpred results which has 8% gaps with 32% identities with my protein, hence,3TTJ can be selected as the best ID which was subjected to hex docking with optimized Ligand (1PCI).

Disorder Prediction Methods: RONN (Regional Order Neural Network):
Disorder prediction results for rage Disordered regions: 1 - 1, 279 - 372, 387 - 414, 417 – 418

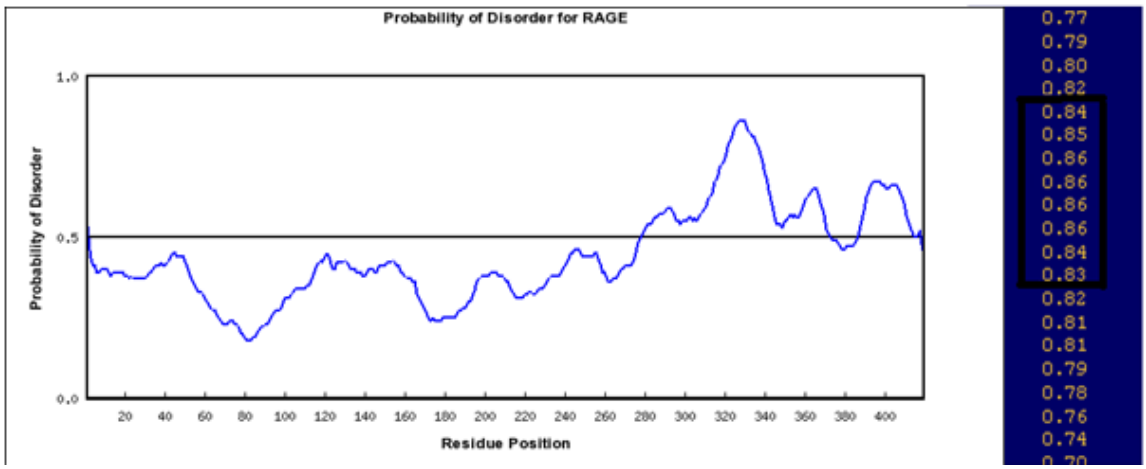


Fig.3. Probability of disorder for RAGE.

```
1     MKNYKAIGKI GEGTFSEVMK MQSLRDGNYY ACKQMKQRFE SIEQVNNLRE 50
      *
51    IQALRRLNPH PNILMLHEVV FDRKSGSLAL ICELMDMNIY ELIRGRRYPL  100
101   SEKKIMHYMY QLCKSLDHIH RNGIFHRDVK PENILIKQDV LKLGDFGSCR  150
151   SVYSKQPYTE YISTRWYRAP ECLLTDGFYT YKMDLWSAGC VFYEIASLQP  200
201   LFPGVNELDQ ISKIHDVIGT PAQKILTKFK QSRAMNFDFP FKKGSGIPLL  250
251   TTNLSPQCLS LLHAMVAYDP DERIAAHQAL QHPYFQEQRK TEKRALGSHR  300
                                  ** ********************
301   KAGFPEHPVA PEPLSNSCQI SKEGRKQKQS LKQEEDRPKR RGPAYVMELP  350
      ********** ********** ********** **********
351   KLKLSGVVRL SSYSSPTLQS VLGSGTNGRV PVLRPLKCIP ASKKTDPQKD  400
      ********** ********** **********
401   LKPAPQQCRL PTIVRKGGR
      ********** ****  **
```

****** => **regions which are prone to disorder.**

Energy Optimization Using SYBYL: Ligand (1PCI)
Energy Optimization:

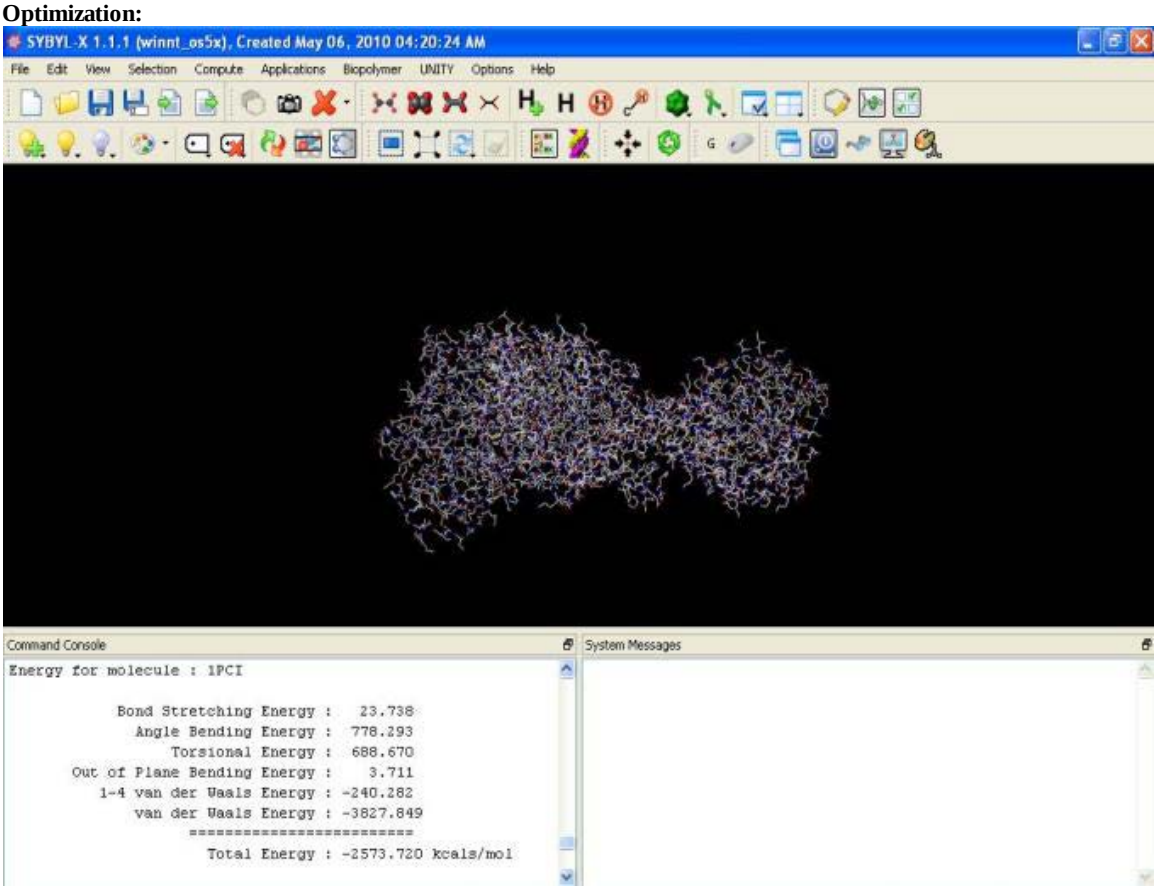


Fig.4. Energy optimization of 1PCI ligand.
Optimized energy = -2573.720 kcal/mol.
Receptor (3TTJ) energy optimization

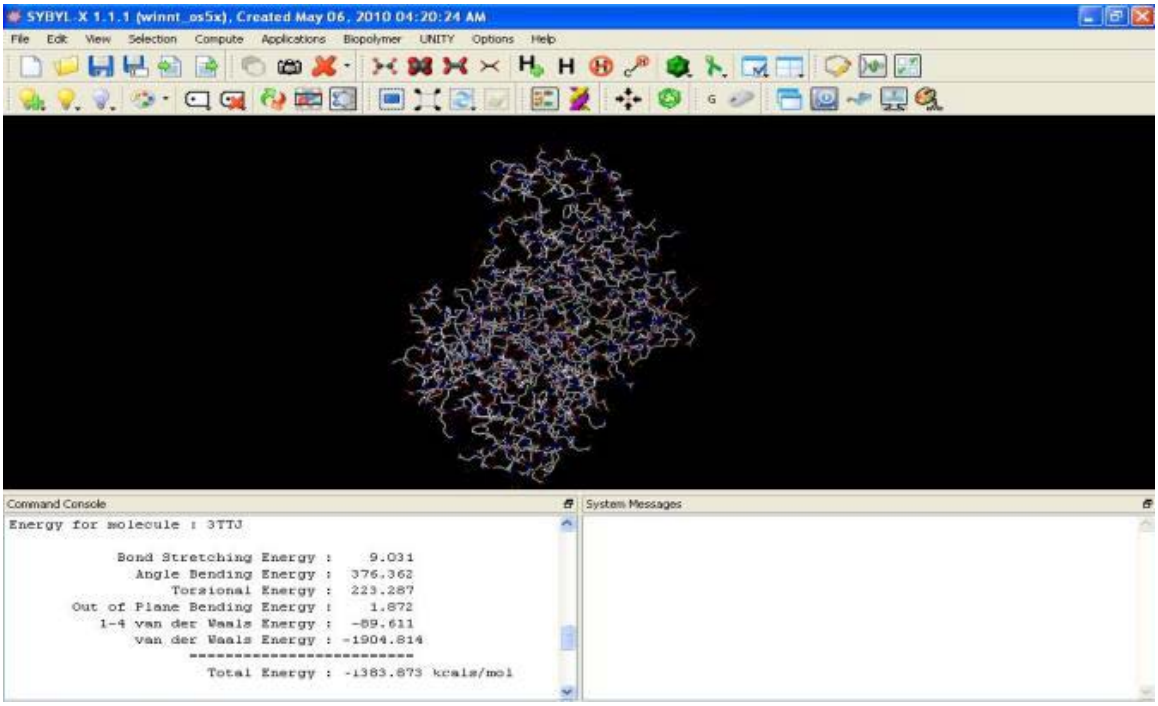


Fig.5.energy optimization of 3TTJ receptor.

Optimized energy = -1363.573 kcal/mol After optimization by SYBYL, optimized ligand (1PCI) and Receptor (3TTJ) are then subjected to Hex docking.

Hex Docking:

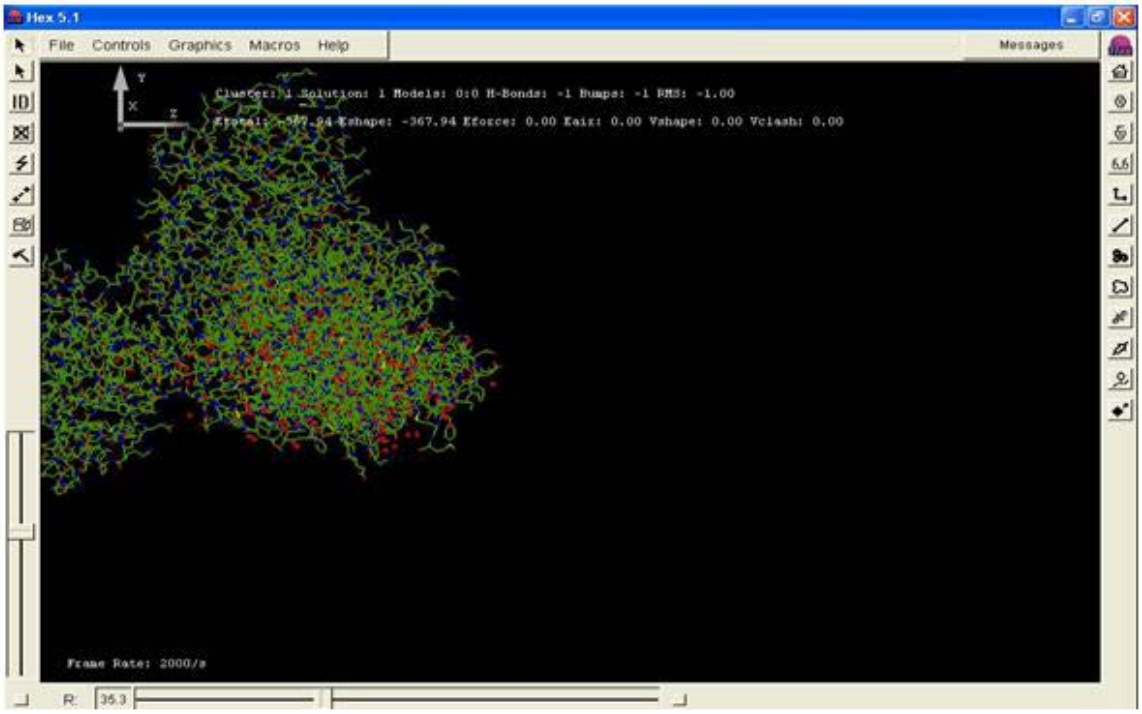


Fig.6. Binding of ligand (Bromelain) and receptor (RAGE) by hex docking.

Hex results showed binding analysis of ligand (Bromelain) and receptor (RAGE). Energy was found to be -367.94 kcal/mol. As the binding energy was found to be very less, it is inferred that RAGE acts a good receptor for docking activity of Bromelain which provides evidence for Bromelain's anti-cancer activity. RAGE presents an important possible target for Bromelain. RAGE is a multi-ligand receptor expressed by many cell types, including cancer. It is regulating activation of NF-Kappa B and its target genes which is stimulated proliferation. We speculate that proteolytic degradation of RAGE could be among the mediators of Bromelain effects. RAGE degradation could potentially ensure cellular AGE protection as well as mediation of Bromelain effects on NF-Kappa B and its targets.

IV. CONCLUSION

The anti-cancerous activity of Bromelain as a ligand and RAGE as receptor was analyzed using in silico studies. And by hex docking, it found that the optimization energy of binding of Ligand (Bromelain) and receptor (RAGE) was -367.94 kcal/mol. As the binding energy was found to be very less, it is inferred that RAGE acts as a good receptor for docking activity of Bromelain which provides evidence for Bromelain's anti-cancer activity.

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