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Research Article

IN SILICO DOCKING ANALYSIS OF THE COMPOUNDS OF *ORTHOSIPHON STAMINEUS* FOR THE ANTICANCER ACTIVITY

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ABSTRACT

The aim of the present study is to investigate the inhibitory activity of the compounds of *Orthosiphon stamineus* on cancer by molecular docking studies and to analyse the ADME/T properties of the compounds. Compound 1- Norstaminolactone A,Compound 2- Norstaminone A, Compound 3- Orthosiphol B, Compound 4- Orthosiphol Q, Compound 5- Orthosiphonone A were isolated from the leaves of *Orthosiphon stamineus*. Compounds 1 to 5 were used for docking on PTK6 (protein tyrosine kinase 6), Oncogene protein (H- Ras GTPase). Glide docking uses the assumption of a rigid receptor, although scaling of Vander Waals radii of nonpolar atoms, which decreases penalties for close contacts, can be used to model a slight "give" in the receptor and/or ligand. Docking studies of designed compounds were carried out using GLIDE (Grid-based Ligand Docking with Energetics) module version 5.9. Schrödinger, LLC, New York, NY, 2013. The software package running on multi-processor Linux PC. GLIDE has previously been validated & applied successfully to predict the binding orientation of many ligands. The docking results showed that the leaves of *Orthosiphon stamineus* are potential for anticancer activity by inhibiting tyrosine kinase protein and oncogene protein. Norstaminolactone A, Norstaminone A, Orthosiphol B and Orthosiphonone A have shown excellent anticancer activity with better ADMET parameters.

Keywords: *Orthosiphon stamineus*, Anticancer, In-silico docking, PTK6, H- Ras GTPase

INTRODUCTION

Cancers are a group of diseases that are characterized by the uncontrolled and proliferative growth of abnormal cells. Such growths can lead to death. Cancer can be caused by both external factors like consumption of tobacco, exposure to chemicals, harmful radiations, infectious organisms, inherited mutations, hormones, and other mutations occurring from normal body metabolism. Cancer treatment depends on the type of cancer, the stage of the cancer (how much it has spread), age, health status, and all other personal characteristics. There is no single treatment for cancer, and patients often receive a combination of therapies and palliative care. Treatments usually fall into one of the following categories: surgery, radiation, chemotherapy, immunotherapy, hormone therapy, or gene therapy. Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world. Natural phytochemicals derived from medicinal plants have gained significant recognition in the potential management of several human clinical conditions, including cancer Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants [9]. More than 3000 plants worldwide have been reported to possess anticancer properties. Therefore, this study was planned to investigate the inhibitory activity of the compounds of *Orthosiphon stamineus* on cancer by molecular docking studies and to analyse the ADME/T properties of the compounds

Protein tyrosine kinase 6 (PTK6, also called BRK) is an intracellular tyrosine kinase expressed in the majority of human breast tumors and breast cancer cell lines, but its expression has not been reported in normal mammary gland. PTK6 activates signal transducer and activator of transcription 3 (STAT3), and active STAT3 was detected in PTK6-positive mammary gland epithelial cells. Protein tyrosine kinase 6 (also called breast tumor kinase or BRK) is a tyrosine kinase that promotes growth factor signaling, and proliferation, migration and survival of breast cancer cells[14]. It was identified in human metastatic breast cancer and is overexpressed in the majority of human breast cancers and in most breast tumor cell lines Functions of PTK6 in normal epithelia are distinct from its roles in cancer. PTK6 is expressed throughout the alimentary canal and in the skin in differentiated epithelial cells, and has been shown to promote differentiation of small intestinal enterocytes and keratinocytes. [10,11,12]

GTPase HRas also known as transforming protein p21 is an enzyme that in humans is encoded by the *HRAS* gene. HRAS has been shown to be a proto-oncogene. When mutated, proto-oncogenes have the potential to cause normal cells to become cancerous. Some gene mutations are acquired during a person's lifetime and are present only in certain cells. These changes are called somatic mutations and are not inherited. Somatic mutations in the HRAS gene in bladder cells have been associated with bladder cancer. One specific mutation has been identified in a significant percentage of bladder tumors; this mutation substitutes one protein building block (amino acid) for another amino acid in the HRAS protein. This overactive protein directs the cell to grow and divide in the absence of outside signals, leading to uncontrolled cell division and the formation

of a tumor. Mutations in the HRAS gene also have been associated with the progression of bladder cancer and an increased risk of tumor recurrence after treatment [13]

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation may be used to predict the strength of binding affinity between two molecules using, scoring functions. The associations between biological molecules such as proteins, nucleic acids, carbohydrates, lipids etc play a central role in transduction. The relative orientation of the two interacting partners may affect the type of signal produced like agonism and antagonism. Therefore, docking is useful for predicting both the strength and type of signal produced. Docking is mainly used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking. In this work we have performed the molecular docking studies of various compounds isolated from the leaves of *Orthosiphon stamineus* and compared the negative binding energy of the docked complex of the protein and the isolated compounds.

Orthosiphon stamineus Benth known as kidney tea or java tea, is a genus of plants in the Lamiaceae family and very popular in Southeast Asian folk medicine, where it is used extensively to treat rheumatoid diseases, diabetes, hypertension, tonsillitis, epilepsy, menstrual disorder, gonorrhea, syphilis, renal calculus, gallstone, lithiasis, edema, eruptive fever, influenza, hepatitis, and jaundice. Its leaves were introduced to Europe and Japan as a health tea. O. stamineus is very well known for its diuretic effect, which is stronger than most other natural diuretics. Many compounds were isolated from this plant used for different biological activities.A Bioactive compounds has been already reported from the leaves of *Orthosiphon stamineus* [1,2] and based on the extensive medicinal claims of leaves of *Orthosiphon stamineus* for the Anticancer activity, the aim of the present study was to investigate the inhibitory activity of the compounds on cancer by molecular docking studies and to analyze the ADME/T properties of the compounds such as Norstaminolactone A, Norstaminone A, Orthosiphol B, Orthosiphol Q and Orthosiphonone were used for docking on PTK6 (protein tyrosine kinase 6), Oncogene protein (H- Ras GTPase to confirm the therapeutic effect of the leaves of this plant.

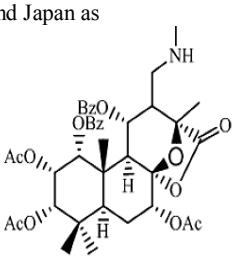
MATERIALS AND METHODS

Software's handled [3,4,5,6]

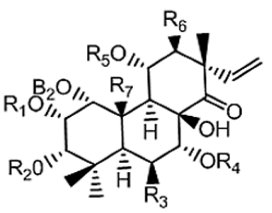
1. GLIDE module version 5.9., mastero 9.4
2. Quik prop -3.6 -Schrödinger, LLC, New York, NY, 2013- docking
3. Swiss PDB viewer-4.04 – protein viewer
4. Pymol viewer 1.3 – image viewer
5. Marveen sketch 5.5 – drawing ligand structure

Ligand structure: The chemical structure of each ligand was drawn using build module**.**

Compound 1- Norstaminolactone A
Compound 2- Norstaminone A
Compound 3- Orthosiphol B
Compound 4- Orthosiphol Q
Compound 5- Orthosiphonone

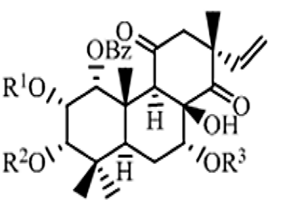


Norstaminolactone A



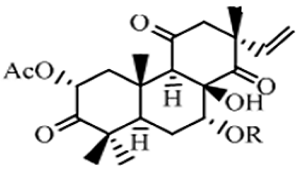
R_1=R_4= Ac; R_2=R_3=R_6=R_7= H; R_5=Bz

Orthosiphol B



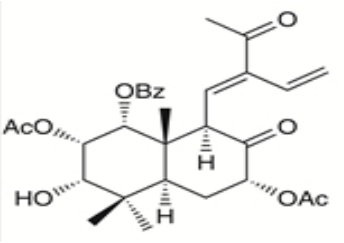
R_1=R_3=Ac ; R_2=Bz

Orthosiphonone A



R=Ac

Orthosiphol Q



Norstaminone A

Docking Procedure

Docking studies of these compounds were performed using the suggested proteins obtained from the RCSB Protein Data Bank, http://www.rcsb.org/pdb.

Experiments were performed using the program GLIDE (Grid-based Ligand Docking with Energetics) module version 5.9, Schrödinger, LLC, New York, NY, 2013 (Schrodinger Inc.). Coordinates of the full-length substrate-complexed dimmer were prepared for Glide 5.9 calculations by running the protein preparation wizard. The p-prep script produces a new receptor file in which all residues are neutralized except those that are relatively close to the ligand (if the protein is complexed with a ligand) or form salt bridges. The impref script runs a series of restrained impact energy minimizations using the Impact utility. Minimizations were run until the average root mean square deviation (rmsd) of the non-hydrogen atoms reached 0.3Å.

Glide uses two boxes that share a common center to organize its calculations: a larger enclosing box and a smaller binding box. The grids themselves are calculated within the space defined by the enclosing box. The binding box defines the space through which the center of the defined ligand will be allowed to move during docking calculations. It provides a measure of the effective size of the search space. The only requirement on the enclosing box is that it be large enough to contain all ligand atoms, even when the ligand center is placed at an edge or vertex of the binding box. Grid files were generated using the cocrystallized ligand at the center of the two boxes.

The size of the binding box was set at 20 Å in order to explore a large region of the protein. The three-dimensional structures of the compounds were constructed using the Maestro interface. The initial geometry of the structures was optimized using the OPLS-2005 force field performing 1000 steps of conjugate gradient minimization. The compounds were subjected to flexible docking using the pre-computed grid files. For each compound the 100 top-scored poses were saved and analyzed.

QikProp Analysis [7,8]

QikProp efficiently evaluates pharmaceutically relevant properties for over half a million compounds per hour, making it an indispensable lead generation and lead optimization tool. Accurate prediction of Absorption, Distribution, Metabolism, Elimination (ADME) properties prior to expensive experimental procedures, such as High Throughput Screening (HTS), can eliminate unnecessary testing on compounds that will ultimately fail; ADME prediction can also be used to focus lead optimization efforts to enhance the desired properties of a given compound.

RESULTS AND DISCUSSION

In this study we have selected a set of 5 compounds of *Orthosiphon stamineus* and performed docking simulations in order to identify their binding energy and binding affinity towards protein tyrosine kinase 6 and oncogene protein and tested their ADME and toxicity profiles using QikProp tool.

The ADME properties of the ligands were predicted using QikProp. The compounds prepared were subjected to drug-likeness filter. The acceptance criteria of the filter includes Molecular weight (< 500), QPPMDCK (500 good, Q Plog BB (-3.0 to 1.2) , Donar HB (0-6) , Metabolism (1-8) , Accept HB (2-20), Log P Value o/w (-2.0 to 6.5) , CNS (-2 to +2) , % Human Oral Absorption (80% high < 25-poor, > 500-Good), PSA (7-200), Q P log S (-6.5 to 0.5), Violation of Rule Of Three (Max 3). All the ligands confirmed to the above mentioned acceptance criteria and they were evaluated for docking using GLIDE software and the results are presented in **table 2**.

Among all the 5 compounds, the docking analysis reports showed that the compound 1 and 4 isolated from the plant does not had any interaction with the enzyme protein tyrosine kinase 6. The results of the docking analysis are given in the **table 1.** Compounds 5 and 2 showed maximum inhibitions with the protein tyrosine kinase (**Figures 1,2 &3**) and compounds 1 and 3 showed maximum inhibition with oncogene protein (**Table 3 & Figures 4-8**). Binding energies in the protein-ligand interactions explain how fit the ligand binds with target protein. Examination of the binding interactions of the ligand helps in elucidating the and appropriate structural features of ligand which increase the binding affinity and therapeutic efficacy.

Table 1: Summary of glide results of the compounds against protein tyrosine kinase 6 for anticancer activity

PARAMETERS	CP 2	CP 3	CP 5
Glide score	-3.363527	-1.894231	-2.631375
Glide energy	-30.446799	-37.019307	-30.251442
Docking score	-3.363527	-1.894231	-2.631375

Table 2: Pharmacokinetic parameters with their optimum range important for CNS activity and Oral Bioavailability obtained by Qikprop tool

PARAMETERS	CP1	CP2	CP3	CP4	CP5
Molecular Weight (< 500)	367.569	550.691	339.364	456.707	438.517
QPPMDCK (<25-Poor, > 500 Good)	440.349	67.421	361.221	254.340	474.96
Violation of Rule of Three (Max 3)	0	0	0	0	0
Donor HB (0-6)	2	1	2	1	1
Metabolism (1-8)	2	3	2	4	3
Accept HB (2-20)	14.95	11.70	15.25	13.55	15.55
Log P Value O/W (-2.0 TO 6.5)	3.766	3.354	3.902	0.894	3.844
CNS (-2 TO +2)	-2	-2	-2	-2	-2
% Human Oral Absorption (80% High <25% Poor)	58.024	72.989	75.309	81.09	76.936
N and O (2-15)	13	9	11	8	11
Violation of Rule of Five (Max 4)	2	1	2	0	2
QP PCACO (< 25-Poor, > 500-Good)	89.607	158.204	747.556	540.475	963.011
PSA (7-200)	184.25	164.716	123.903	117.223	140.231
Q P log S (-6.5 TO 0.5)	-4.971	-5.123	-4.688	-2.661	-4.769
Q P log BB (-3.0 TO 1.2)	-1.179	-1.773	-1.245	-1.041	-1.123

Table 3: Summary of glide results of the compounds against C-H-RAS P21 protein (oncogene protein) for anticancer activity

PARAMETERS	CP1	CP2	CP3	CP4	CP5
Glide score	-6.686493	-4.205072	-6.970526	-5.222854	-5.420907
Glide energy	-64.472291	-53.242665	-46.402815	-33.88193	-52.723287
Docking score	-6.686493	-4.205072	-6.970526	-5.222854	-5.420907

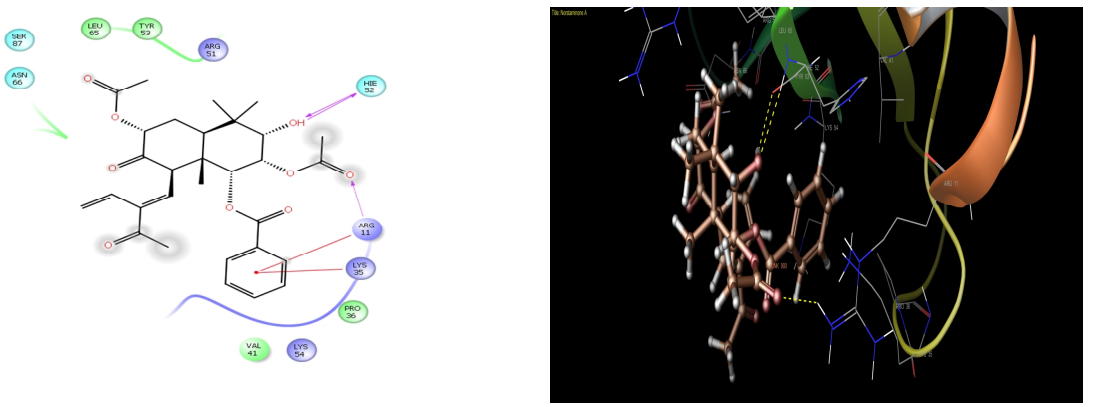


Figure 1: Docking interaction of compound 2 with protein tyrosine kinase

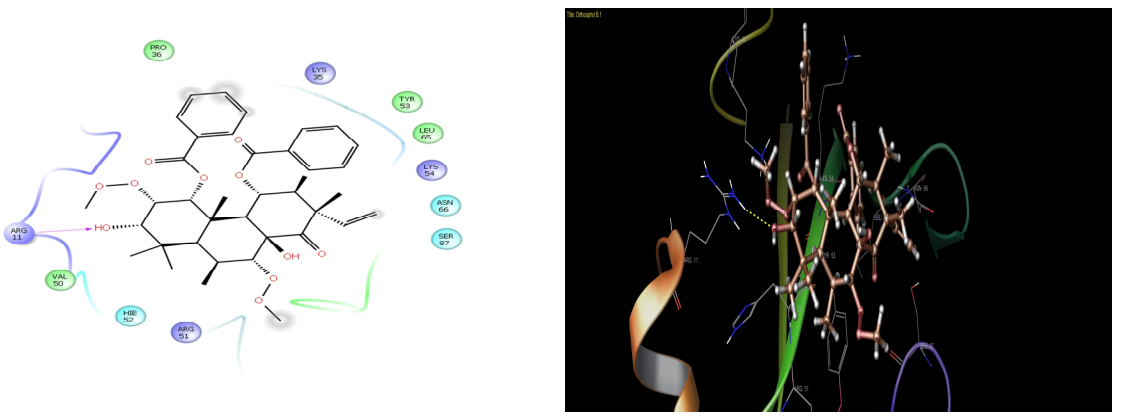


Figure 2: Docking interaction of compound 3 with protein tyrosine kinase

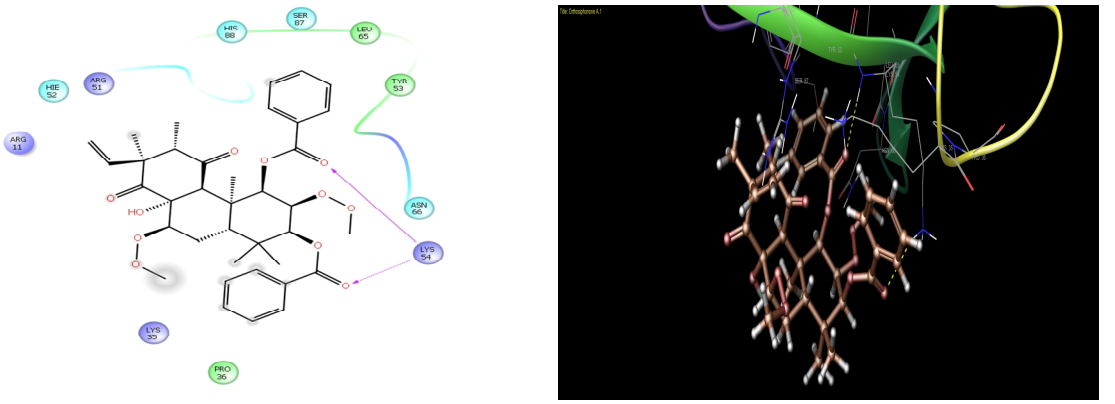


Figure 3: Docking interaction of compound 5 with protein tyrosine kinase 6

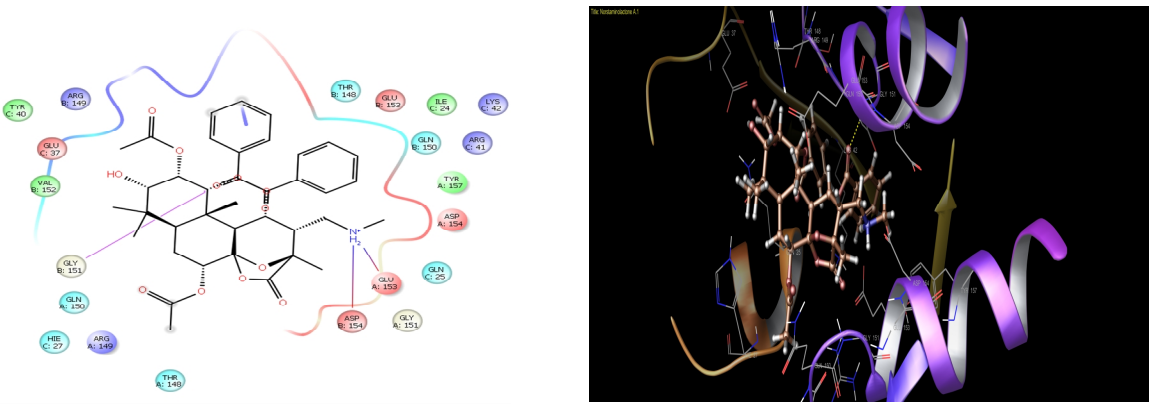


Figure 4: docking interaction of compound 1 with H- Ras GTPase for anticancer activity

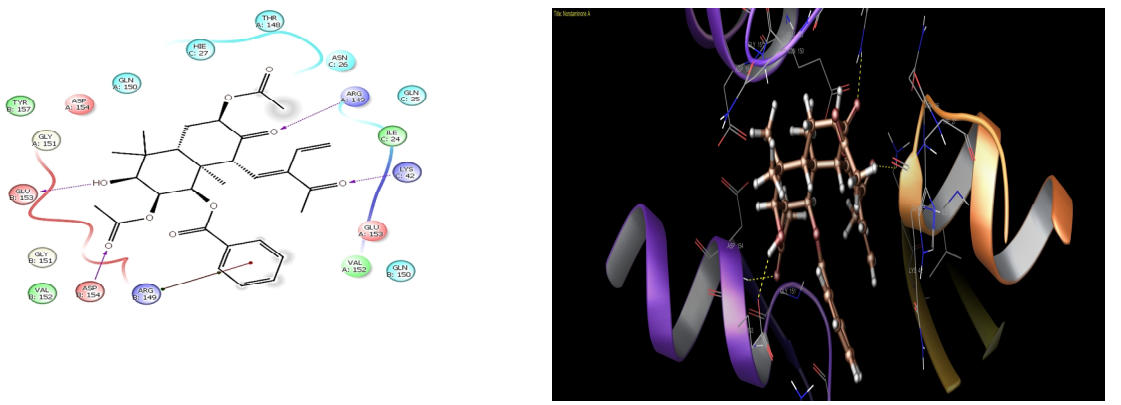


Figure 5: docking interaction of compound 2 with H- Ras GTPase for anticancer activity

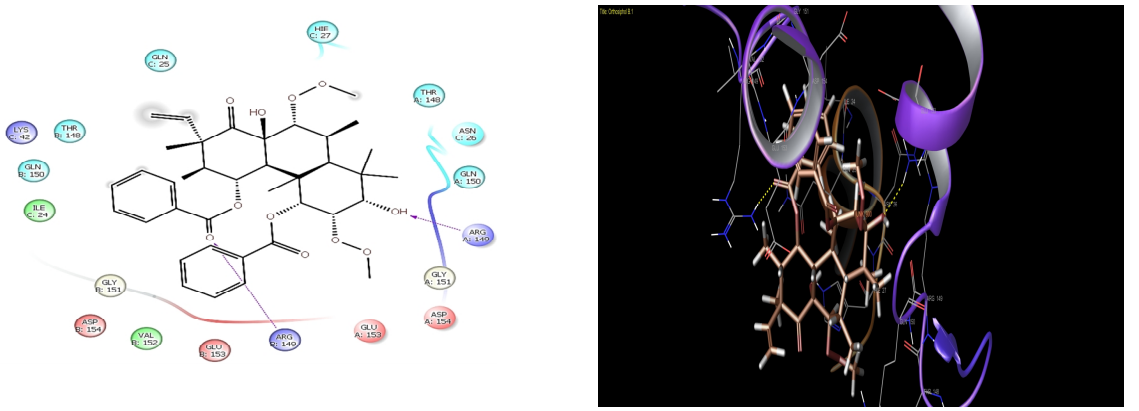


Figure 6: docking interaction of compound 3 with H- Ras GTPase for anticancer activity

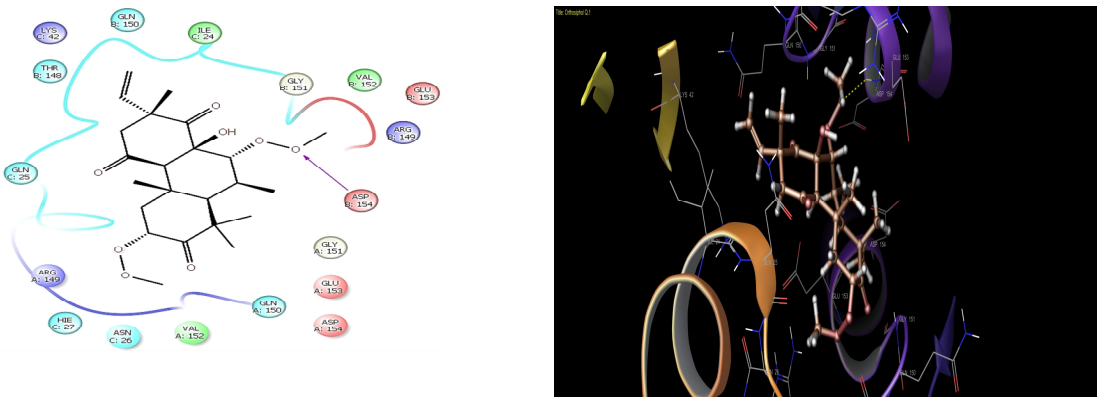


Figure 7: docking interaction of compound 4 with H- Ras GTPase for anticancer activity

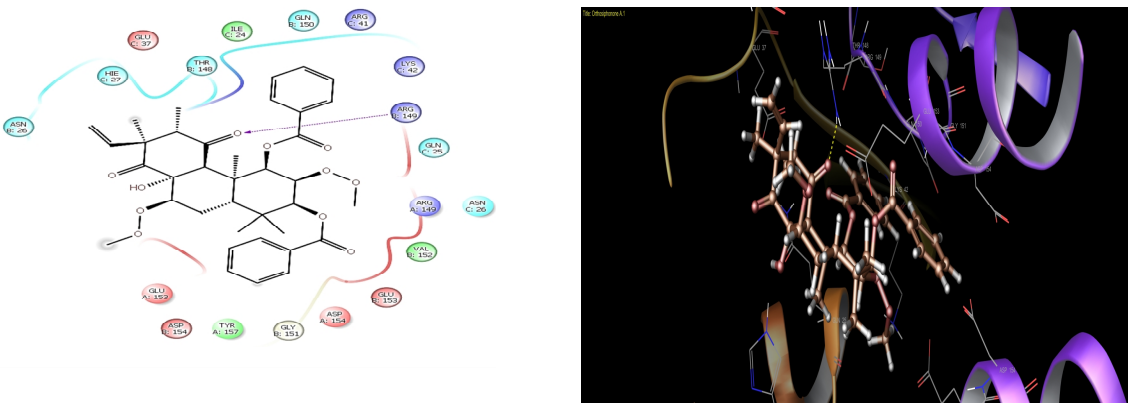


Figure 8: Docking interaction of compound 5 with H- Ras GTPase for anticancer activity

CONCLUSION

The docking results showed that the leaves of *Orthosiphon stamineus* are potential for anticancer activity by inhibiting tyrosine kinase protein and oncogene protein. Norstaminolactone A, Norstaminone A, Orthosiphol B and Orthosiphonone A have shown excellent anticancer activity with better ADMET parameters. Isolation of Orthosiphonone A and Norstaminone A, beneficial for Breast cancer and isolation of Norstaminolactone A and Orthosiphol B beneficial for Bladder cancer.

REFERENCES

1. Sumaryono W., ProkschP.,Wray V., Witte L., L., Hartmaan T., Qulatative and Quantitative analysis of the phenolic constituents from Orthosiphon aristatus. Planta medica:1991;57:176-180
2. M Amzad Hossain, S .M. Salehuddin and Zhari Ismail Isolation and Characterization of a new poly hydroxy Flavone from the leaves of *Orthosiphon stamineus*, Indian Journal of Natural Products: 2007:23(4):3-7
3. Halgren, T. A, Murphy, R. B, Friesner, R. A, Beard, H. S, Frye, L. L, Pollard, W. T, Banks, J.L Glide, A New Approach for Rapid, Accurate Docking and Scoring. 2.

Enrichment Factors in Database Screening, Journal of Medicinal Chemistry: 2004**:**47: 1750-1759
4. Sherman W, Day T, Jacobson M. P, Friesner R. A, Farid, R, Novel Procedure for Modelling Ligand / Receptor Induced Fit Effects, Journal of Medicinal Chemistry : 2006:49: 534-553.
5. Golke H, Hendlich M, Kelbe G, Knowledge-Based Scoring Function to Predict Protein- Ligand Interactions .Journal of Molecular Biology:2000:295: 337-356.
6. Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes: Journal of Medicinal Chemistry: 2006: 4:, 6177-6196
7. Jorgensen W.L, Duffy E.M, Prediction of Drug solubility from Monte Carlo simulations, Bioorganic Medicinal Chemistry Letters: 2000:10:1155-1158.
8. Lipinski CA, Lombardo F, Dominy BM, Feeney PJ, Experimental and Computational approaches to estimate solubility and permeability in Drug Discovery and Development setting, Advance Drug Delivery Reviews:2001: 46, 3-26
9. Kaur R, Kapoor K and Kaur H. Plants as a source of anticancer agents. Journal of Natural Products and Plant Resourources;2011: 1 (1): 119-124.
10. Harvey AJ, Crompton MR. The Brk protein tyrosine kinase as a therapeutic target in cancer: opportunities and challenges. Anticancer Drugs :2004; 15: 107–111. |
11. Brauer PM, Tyner AL. Building a better understanding of the intracellular tyrosine kinase PTK6 - BRK by BRK. Biochimica et Biophysica Acta ;2010; 1806: 66–73.
12. Ostrander JH, Daniel AR, Lange CA. Brk/PTK6 signaling in normal and cancer cell models. Current Opinion in Pharmacology ;2010; 10: 662–669.
13. Mechanisms of Disease: genetic and epigenetic alterations that drive bladder cancer *;* Nature Reviews Urology ;2005;2;502-510
14. R. Lakshmi et al. Breast cancer risk factors: preventable and non-preventable, Int Res J Pharm 2012:3(10):48-52.

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