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Research Article

MOLECULAR DOCKING OF COMPONENTS FROM THE EXTRACTS OF ENDOPHYTIC BACTERIA OF *CISSUS QUADRANGULARIS* AGAINST AURORA B KINASE

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ABSTRACT

Compounds that inhibit the activity of Aurora kinase enzyme are potential drug candidates for a variety of cancers including osteosarcoma. Endophytes of medicinal plants have potential to produce several anticancer compounds having pharmaceutical applications. *Cissus quadrangularis* Linn. is a versatile medicinal plant with various therapeutic properties including bone fracture healing. Endophytes of this plant may be a promising source of anticancer compounds. The compounds present in the extracellular extract of endophytic bacteria predicted by GCMS and the compound VX-680 (Tozasertib) were subjected to docking against Aurora B kinase using AutoDock 4.2. Molecular docking studies has revealed that, among the compounds docked, five of them and a known Aurora B Kinase inhibitor VX-680, hydrogen bonded with the amino acid ALA173 in the active site of the enzyme.VX-680; Dl-leucine, n-dl-leucyl-; Acetophenone 4'-methoxy; Benzaldehyde, 4-methoxy; 3-Nonanol, 1,2;6,7-Diepoxy-3,7-Dimethyl-Acetate; Cyclopropane,1,2-dibutyl and 2-Myristynoyl-Glycinamide; bind to Aurora B Kinase (2VRX) with a binding energy of, -8.53, -4.68, -4.65, -4.34, -4.0, -3.93 and -3.75 kcal mol^{-1} respectively. Thus, virtual studies on the endophytic compounds, reveal that these compounds may possess the potential to hinder the activity of Aurora B kinase and thus can contribute as lead compounds in developing therapeutic agents for cancer.

Keywords: Osteosarcoma, 2VRX, endophytic bacteria, Aurora kinase, Autodock 4.2.

1. INTRODUCTION

Cancer prevails as one of the leading causes of death worldwide, in spite of therapy. Although developments have been made in the treatment of cancers, it is still a highly prevalent global problem. The limitations and downsides of conventional chemotherapy and radiotherapy including drug resistance and serious side effects, have persuaded the search for new antitumor agents with greater virtue and fewer ruinous effects. Many of the drugs or combinations of drugs are used for chemotherapy and many more are under research. Appropriate use of drugs, targeting the mechanism of proliferation of cancer cells has to be considered [1, 2]. Natural bioactive compounds harnessed from biological sources serve as anticancer agents [3]. The cure of cancer can be enhanced by developing anticancer drugs which are nontoxic to normal cells, have no side effects and those that are effective against many forms of cancer. Endophytes, the plant associated microorganisms are promising sources of compounds for developing anticancer drugs. The anticancer properties of several secondary metabolites from endophytes have been investigated [4]. Endophyte extracts have been considered to be a better option at variance with chemotherapy agents due to their anticancer potential and assumed lower side-effects, as they are less toxic to normal cells. The natural endophyte-derived metabolites have attracted curious scrutiny for being human cancer-chemopreventive compounds and anticancer chemotherapeutic drugs [5].

One of the criteria for selecting the host plant as a source of endophytes is that the plant should have ethnomedicinal importance [6]. *Cissus quadrangularis* Linn. is a succulent plant of Vitaceae family and is used as a medicinal plant since antiquity. The plant is well known

for its bone fracture healing properties. Stem of *Cissus quadrangularis* Linn. is very important part of the plant used in the traditional system of medicines to treat piles, pain in joints, swelling, scurvy, osteoporosis, asthma, cough, hemorrhoids, and gonorrhea. Notable bio-activities such as antimicrobial, antioxidant, antiinflammatory and anticancerous are also cited for this plant extract [7]. The molecular docking studies supported the *in vitro* anticancer activities of *Cissus quadrangularis* Linn [8]. Endophytic fungi and endophytic bacteria from *Cissus quadrangularis* Linn. have been isolated and their extracts have revealed the presence of anticancer agents [9, 10].

Bioinformatics employing modern computation is a rapid and cost-effective tool to screen the plethora of compounds against a therapeutic target. Virtual screening of large number of compounds is done with the help of molecular data banks [11]. Prediction of the correct orientation of the receptor protein with the potential compound of interest and estimation of the binding strength becomes possible with molecular docking studies. Through the virtual screening studies, the compounds that have potential to be used as inhibitors of target enzyme can be predicted by unfolding the binding energy and their mode of interaction at the active site of the target protein. In recent years, molecular docking studies have become fundamental in drug discovery research [12].

Large number of compounds still remains to be screened as it is a limiting step in current researches. Many investigators in this modern era screen the compounds through various docking tools, the results of which support the bioassays either prior to or after docking. Molecular docking method is a part of *in silico* approach that predicts the binding mode and binding affinity of small molecule (query ligand) against a target protein (receptor)[13]. Docking serves as a quick and inexpensive tool for screening large number of compounds in search for novel drug leads with the help of computer and software. This allows compounds to be computationally screened against a target/targets of choice of known structure. Those compounds that are predicted to bind well with the target can be further tested in wet lab for confirming the bioactivity. Thus, docking eases the process of predicting and preliminarily selecting lead molecules among a huge database [14].

There are several molecular docking studies which report the virtual screening of lead molecules that bind to therapeutic targets of cancer and serve as an inhibitor of target protein. Such molecular docking studies provide insights whether the compounds can be used as anticancer drugs. *In silico* study on compounds of endophyte indicated that hexa-hydropyrrolo [1,2-a]pyrazine-1,4-dione and its related compounds: 4,4'-(1,2-dimethyl-1,2-ethanediyl) bis-2,6-piperazinedione and Razoxane could be potent inhibitors against cancer chaperone Hsp90 and therefore can be considered for *in vitro* and *in vivo* analysis towards development of drugs which may act as Hsp90 inhibitors [15]. The compounds (ligands) of an endophyte *Chaetomium* sp. were docked into Human Estrogen Receptor alpha (HERD) as the protein which regulates the breast cancer growth via estradiol-estrogen receptor binding intervention. Two compounds bearing xanthone and two compounds bearing benzonaphtyridinedione scaffolds were selected as virtual hit ligands for HERD leading to the conclusion that these compounds were good to be developed as anti-breast cancer agents [16]. Various endophytic metabolites were virtually screened towards anticancer property. The most active anticancer compound was found to be Phomol with the docking score of -9.778 kcalmol^{-1} [17].

The Aurora kinases are a family of highly conserved serine/threonine kinases that are important for regulating mitosis and their function is to ensure accurate chromosome segregation and cell body division [18-20]. Abnormal expression or activation of Aurora kinases may potentially result in deviant mitosis leading to the development of various cancers. Thus, Aurora kinases are oncogenic drivers [21-23]. Aurora B kinase is over-expressed in several different cancer cells and studies have indicated its possible involvement in transforming normal cells to malignant cells [23-25]. Aurora B kinase has been found to be overexpressed in a wide variety of human tumors including osteosarcoma. These observations suggests that a wide range of cancers as well as osteosarcoma could respond therapeutically to inhibitors of Aurora kinases and hence have kindled the academic and pharmaceutical researchers to discover small molecules that are Aurora kinase inhibitors inducing cell death as anticancer drugs [26-28].

Endophytic bacteria were isolated from *Cissus quadrangularis* Linn. and the compounds present in their extracellular extract were predicted using GCMS [29]. The present work is focused on the molecular docking analysis of these endophytic metabolites predicted by GCMS and VX-680 against Aurora B kinase.

2. MATERIAL AND METHODS

The following six compounds (ligands): 3-Nonanol, 1,2;6,7-Diepoxy-3,7-Dimethyl-Acetate; 2 Myristynoyl-Glycinamide; Dl-leucine, n-dl-leucyl-; Benzaldehyde, 4-

methoxy; Acetophenone 4'-methoxy; Cyclopropane,1,2-dibutyl were subjected to docking analysis. In addition to these a reference compound VX-680 (a known Aurora B kinase inhibitor) was also docked against Aurora B Kinase enzyme.

2.1. Target Protein Structure

The three-dimensional (3D) structure of the Aurora B kinase enzyme was taken from the Protein Data Bank (PDB) database (www.rcsb.pdb). The PDB ID of Aurora B kinase is 2VRX, which is a complex of the enzyme with selective inhibitor compound ZM447439. The structure of Aurora B kinase and the active site region are shown in Fig. 1 and 2 respectively.

2.2. Preparation of compounds structure

The structures of ligands (endophytic bacterial metabolites and VX-680) were retrieved from the Pub Chem database and converted to PDB file format (Fig. 3, 4).

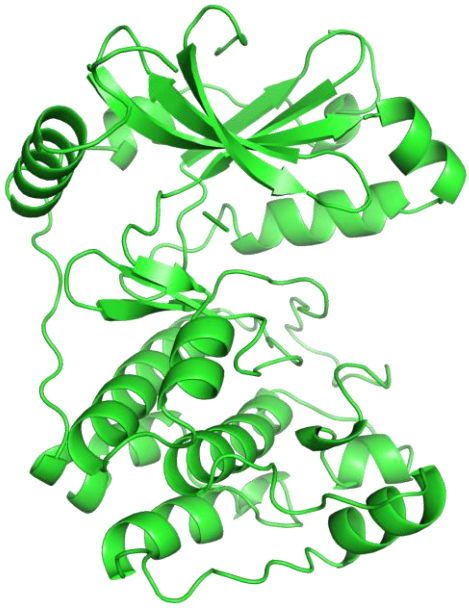

Fig. 1: Aurora B kinase

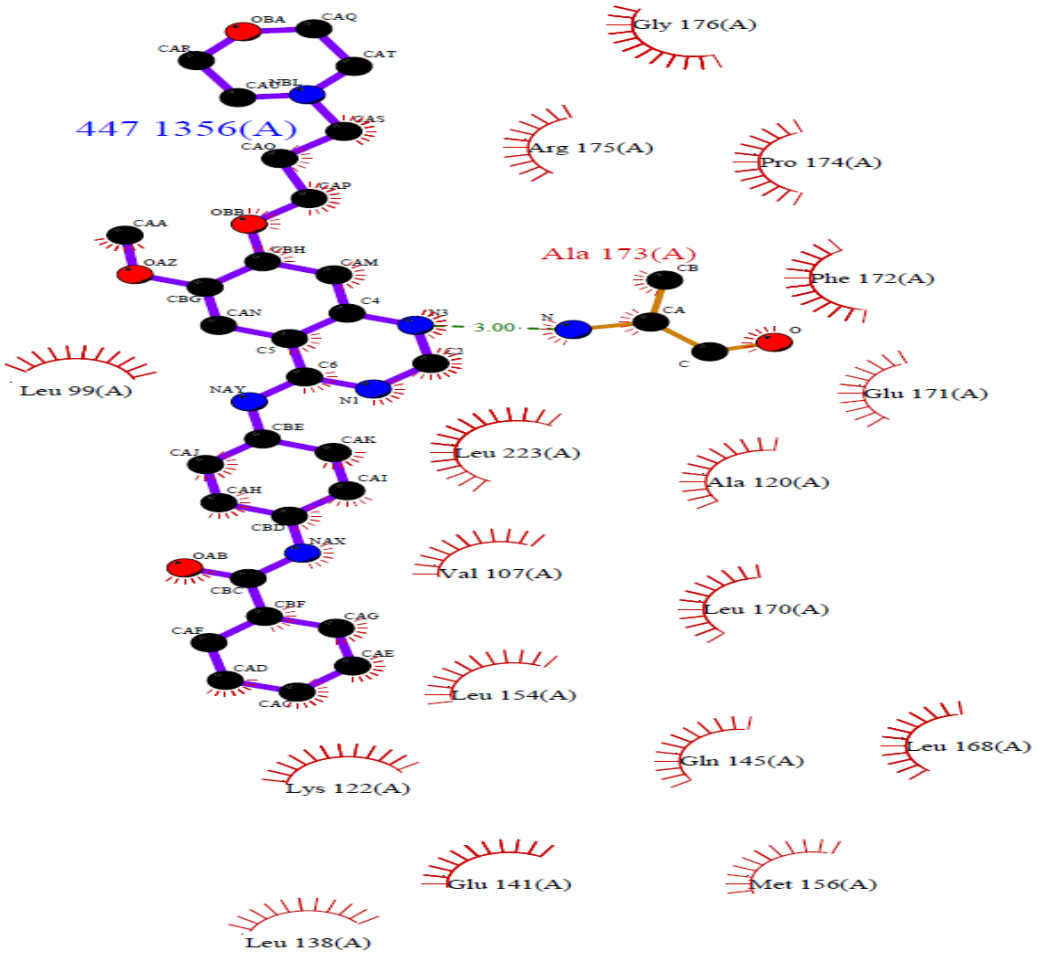

Fig. 2: Aurora B kinase in complex with selective inhibitor ZM447439 showing the active site region (ligPlot)

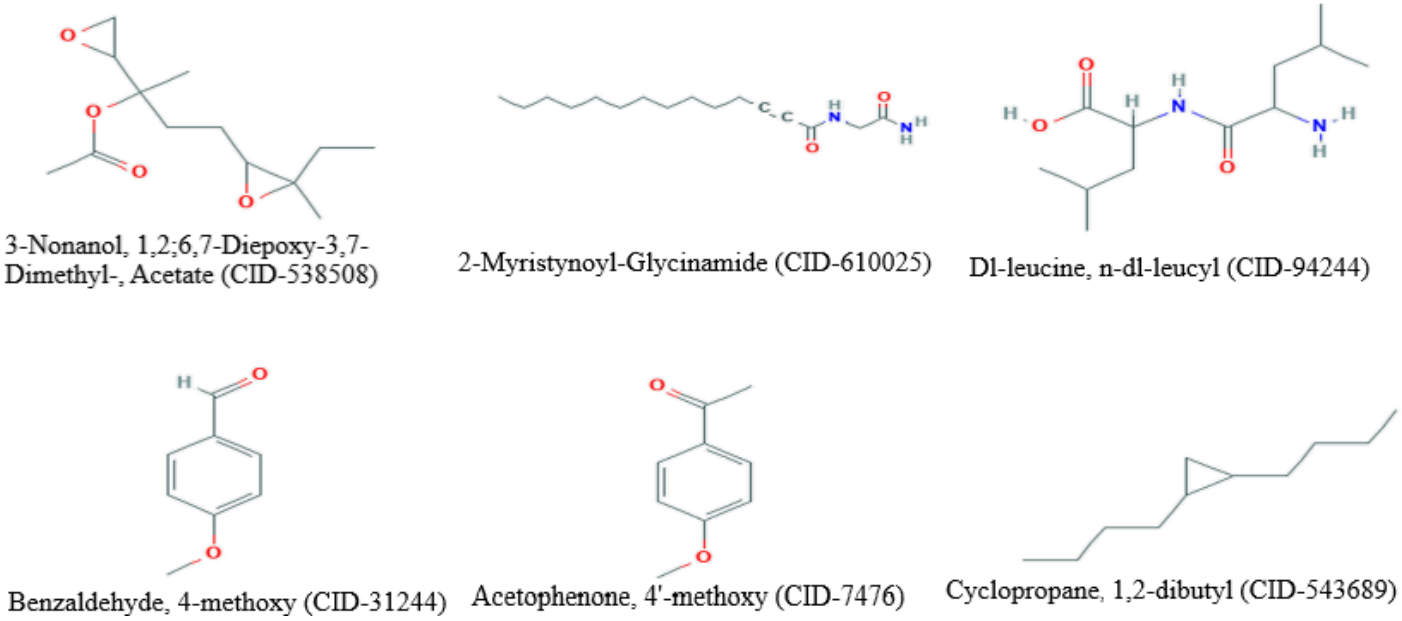

Fig. 3: Structures of metabolites (ligands) present in the extracellular extract of endophytic bacteria

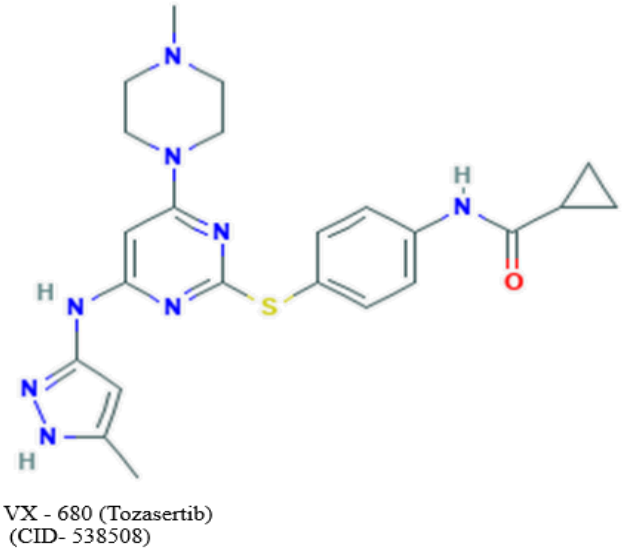

Fig. 4: Structure of VX-680 (ligand)

2.3. Docking studies

The receptor structure of protein Aurora B kinase (PDB ID: 2VRX) was retrieved from the protein data bank and prepared for further study. The co-crystallized ligand molecule was removed from the structure of protein and the missing residues were built manually. All the water molecules were removed and hydrogen atoms were added to the molecule using Pymol [30]. The AutoDock 4.2 program was used for docking calculations [31]. AutoDock uses the Lamarckian genetic algorithm and is regarded as the best method in terms of its ability to deduce the lowest energy structure and the accuracy of its structure predictions [32]. Hydrogen atoms and the active torsions of ligands were assigned using AutoDock tools. The binding site for the receptor structure was created within 5 Å. An autogrid was further employed to generate grid maps around the active site with 50×50×50 points and grid spacing set to 0.375 Å. The default docking parameters were modified. The number of individuals in the population was set to 150, maximum number of energy evaluations was set to 2,500,000, maximum number of generations was set to 27,000, and number of GA (genetic Algorithm) runs was set to 20. The final conformations were clustered and ranked according to the AutoDock scoring function as well as with the knowledge of crucial residues. The binding energy and the binding contacts of each ligand were obtained. Analysis of the docked complexes was done using PyMol [30].

3. RESULTS AND DISCUSSION

The current study focuses on the virtual investigation of natural compounds from the endophytic bacteria of *Cissus quadrangularis* for anticancer property. The compounds in the extracellular extract of endophytic bacteria isolated from *Cissus quadrangularis* were predicted by GCMS and previously reported [29]. Molecular Docking of these compounds (ligands) against the Aurora B kinase was done using AutoDock 4.2

program. It is an automated docking tool which works by Lamarckian Genetic Algorithm. The binding of small molecules to Aurora B kinase (PDB ID: 2VRX) was predicted. A total of 7 compounds including VX-680 as a reference inhibitor compound were docked in the active site of 2VRX. The binding energy, number of hydrogen bonds, residues involved in hydrogen bonding and inhibition constant are tabulated in Table 1. The structure of each ligand bound to the enzyme is shown in figs. 5-11.

The docking interactions of natural endophytic compounds were studied along with the known inhibitor VX-680 that is reported to have activity against Aurora B kinase [33]. All the ligands studied except Cyclopropane, 1,2-dibutyl bound to Ala173 in the active site region of Aurora B kinase enzyme, with considerable binding energy in the same pocket. Ala173, Glu171, Glu177, Lys122 are the critical amino acids present in the template [34, 35].

The compound Dl-leucine, n-dl-leucyl- bind in the active site region of the enzyme with the calculated binding energy -4.68 kcal mol^{-1} and inhibition constant 371.31µM. Acetophenone, 4'-methoxy forms two hydrogen bonds, one with Ala173 and the other with Glu177 and binding energy was predicted as -4.65 $kcalmol^{-1}$and the inhibition constant was found to be 389.26 µM.The binding energy of VX-680 was found to be the least (-8.53 $kcalmol^{-1}$) and the inhibition constant was found to be in nanomoles quantity (561.58 nM). This is in accordance with the fact that VX-680 also known as Tozasertib is a known inhibitor of Aurora B kinase and a drug candidate [36-38].

Table 1: Interactions of various compounds

S. No.	Compound (LIGAND)	Binding energy ($kcalmol^{-1}$)	No. of Hydrogen bonds	Residues involved in hydrogen bond interaction	Inhibition constant
1	3-Nonanol, 1,2;6,7-Diepoxy-3,7-Dimethyl-, Acetate	-4.0	1	Ala173	1.18mM
2	2-Myristynoyl-Glycinamide	-3.75	1	Ala173	1.77mM
3	Dl-leucine, n-dl-leucyl-	-4.68	2	Ala173, Glu177	371.31µM
4	Benzaldehyde, 4-methoxy	-4.34	2	Ala173, Glu177	657.22µM
5	Acetophenone, 4'-methoxy	-4.65	2	Ala173, Glu177	389.26µM
6	Cyclopropane, 1,2-dibutyl	-3.93	Nil	Nil	Nil
7	VX680 (Tozasertib)	-8.53	1	Ala173	561.58nM

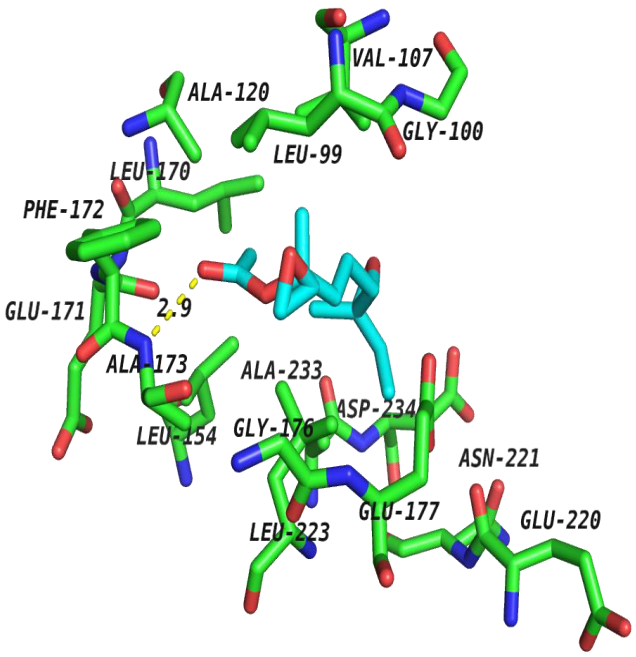

Fig. 5: Interaction of 3-Nonanol, 1,2;6,7-Diepoxy -3,7-Dimethyl-Acetate with Aurora B kinase

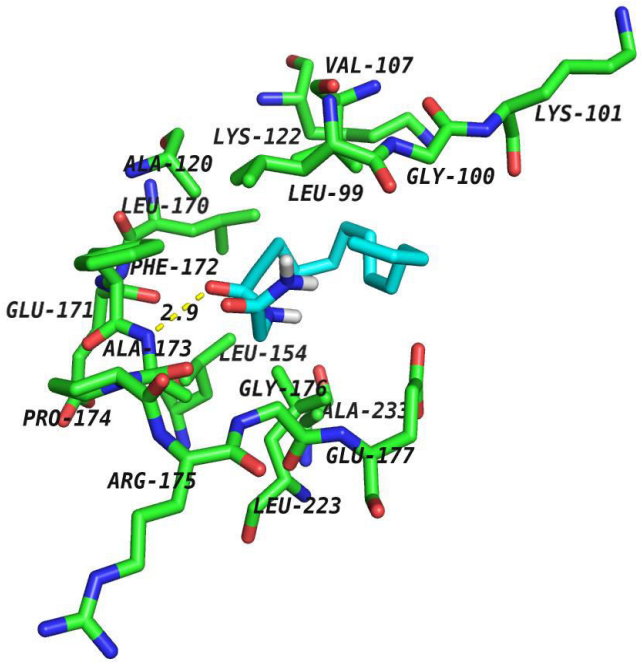

Fig. 6: Interaction of 2-Myristynoyl-Glycina-mide with Aurora B kinase

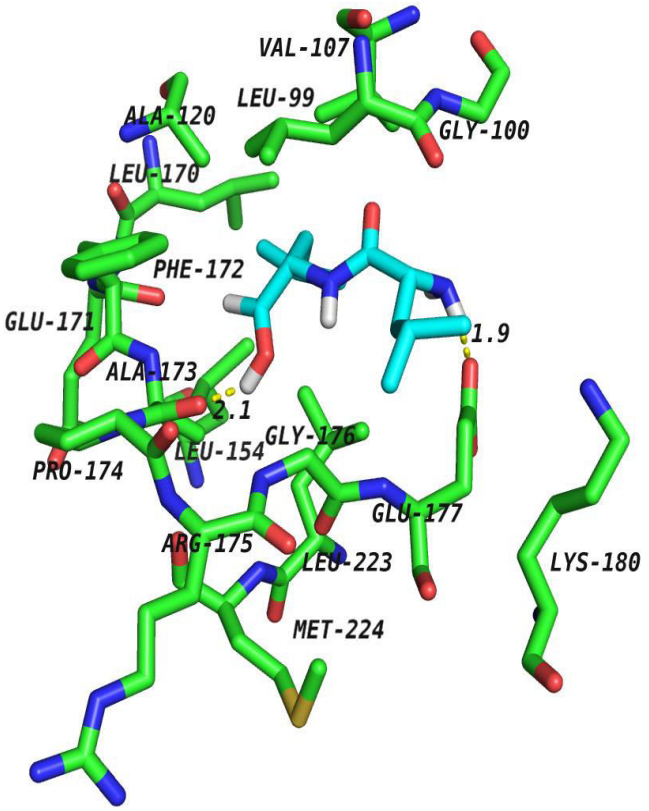

Fig. 7: Interaction of Dl-leucine, n-dl-leucyl- with Aurora B kinase

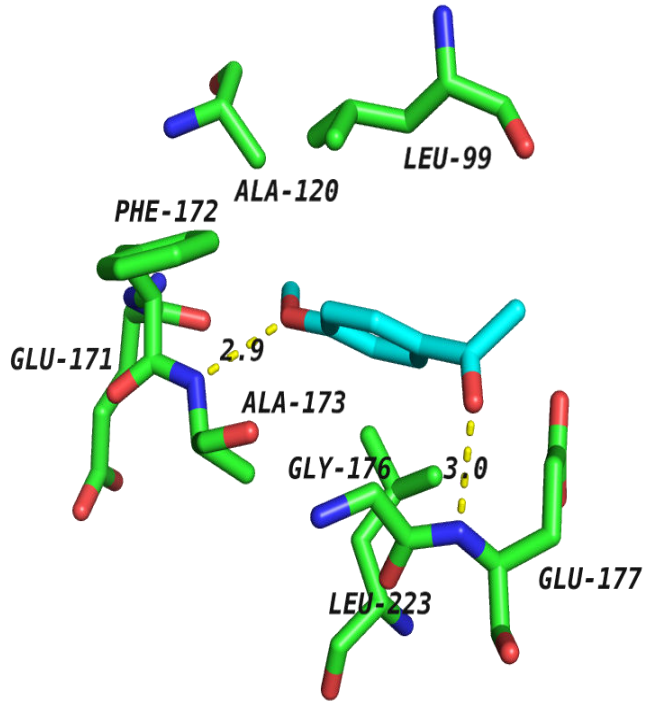

Fig. 9: Interaction of Acetophenone, 4'- methoxy with Aurora B kinase

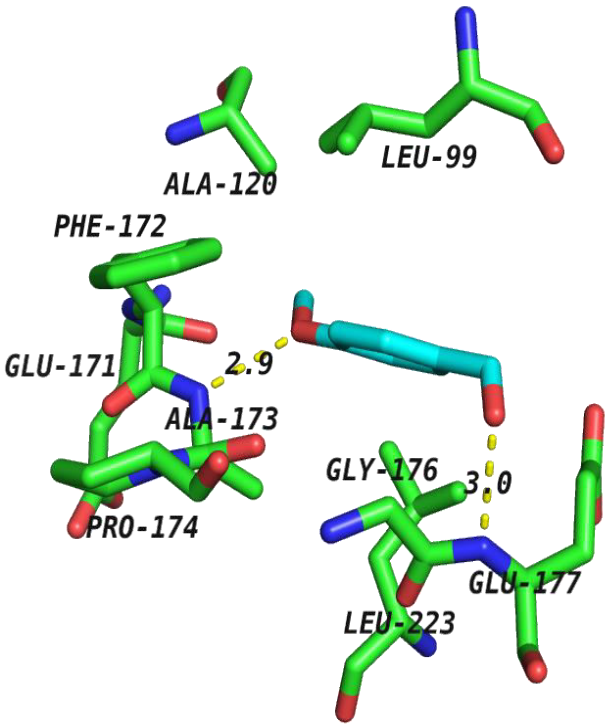

Fig. 8: Interaction of Benzaldehyde, 4-methoxy- with Aurora B kinase

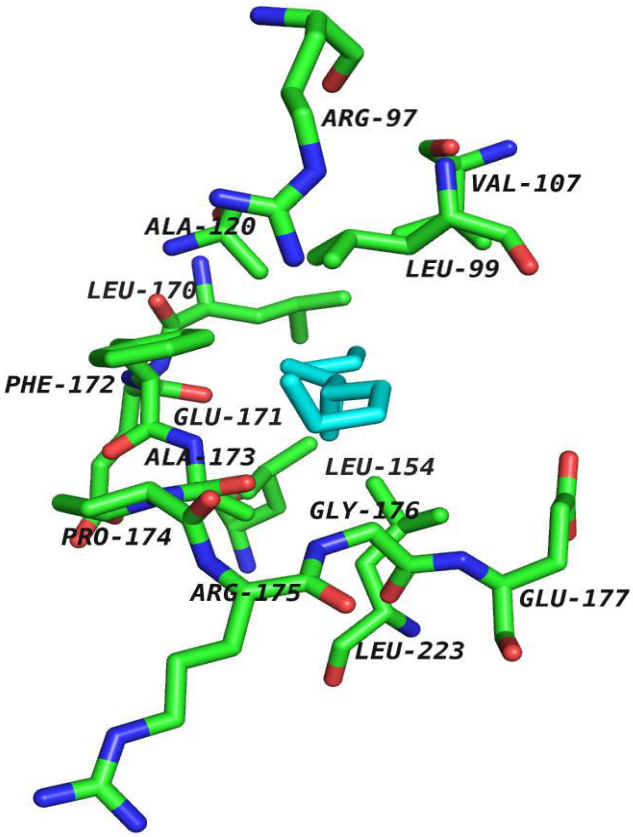

Fig. 10: Interaction of Cyclopropane, 1,2- dibutyl with Aurora B kinase

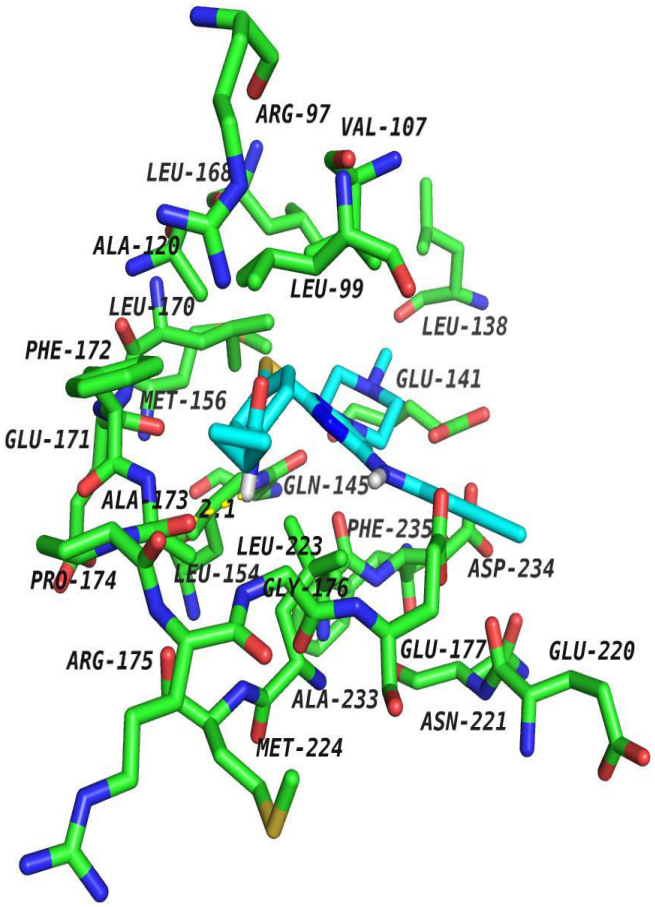

Fig. 11: Interaction of VX-680 with Aurora B kinase

The lower the binding energy higher is the stability of the bound confirmation and effective will be the ligand as a drug candidate. Although the binding energies of the endophytic compounds were greater than that of VX-680 it can still be inferred that the compounds have the ability to bind to target enzyme and the potential to interfere with the activity of Aurora B kinase enzyme. Among those mentioned amino acid residues, interaction with ALA173 was the most prevalent and it was suggested to be important as this interaction was also possessed by the reference ligand. Therefore, compounds having interaction with ALA173 residue with the least binding energy may have the potential to inhibit the target enzyme Aurora B kinase and hence may possess anticancerous activity. The components of the endophytic extracts may be used in combination, to interfere with the Aurora B kinase activity. Synergistic effect of all the potential lead compounds would play promising role in inhibiting the enzyme. Similar docking studies were carried out for set of synthesized compounds against Aurora kinases including 2VRX keeping in view the importance of Aurora kinase inhibitors in cancer treatment [39].

Adult and childhood malignancies are treated with important group of chemotherapeutic agents that have antimitotic activity. In the past few years many new agents are proposed as cell cycle-interacting drugs, some of which have fortunately passed preclinical and clinical validation. Aurora kinase family of enzymes are key regulators of mitosis and cell cycle and hence many drugs have been developed for inhibiting their functions. Aurora kinases represent new promising therapeutic targets in several human tumours. A number of inhibitors of Aurora kinases have been reported. Among these, VX-680 (also referred as MK-0457 or Tozasertib) and ZM447439 are potent small molecule inhibitors of Aurora kinases and have been studied in several human tumours. These are reported to have entered clinical trials. VX-680 and ZM447439 target the ATP-binding site of Aurora kinases and inhibit all three family members; however, their efficacy appears to be mainly associated with the inhibition of Aurora A or Aurora B. Clinical experience with VX-680 and ZM447439 showed that these drugs do not affect human normal cells and that their combination with conventional chemotherapeutic agents may improve the clinical efficacy of standard treatment regimens without increasing collateral toxicity [23, 24, 36, 40-44].

Osteosarcoma (OS), the bone tumour, affects children and adult and is a highly invasive and metastatic rare tumour, which can frequently develop chemoresistance. Introduction of neoadjuvant chemotherapy based on a combination of different drugs has significantly improved the clinical outcome of localized high grade OS. There are patients who poorly respond to the chemotherapeutic treatment and relapse [45, 46]. Thus, novel effective treatment strategies are needed to improve the clinical outcome of OS. Interestingly, Aurora B kinase gene is located on chromosome 17 position 13.1, which has also been found to be amplified in osteosarcoma [47]. *In vitro* analyses carried out by Tavanti *et al.*, have shown that human osteosarcoma cell lines proved to be highly sensitive to VX-680 and ZM447439. These can be new promising drugs of potential clinical usefulness in association with conventional osteosarcoma chemotherapeutic agents [48]. Thus, these evidences show that Aurora B kinase inhibitors can be used to develop therapeutics against many types of cancers including osteosarcoma. The interaction of candidate molecules with their molecular

targets forms the basis for drug development process. Our docking studies support the earlier report by us which documented the inhibition of osteosarcoma cell line Saos2 by the endophytic extract under study [29]. Moreover, the results of this study also support the previous investigation that Benzaldehyde, 4-methoxy found to be present in the extract possesses anticancer activity [29, 49]. Further, these compounds can be checked for its drug likeness properties and toxicity profile. The endophytic bacterial extract could be potential inhibitory source against the crucial receptor Aurora B kinase and hence a promising source of anticancer compounds.

Cissus quadrangularis has been known for its bone setting properties and also reported to possess anticancerous potential [50]. The anticancer activity of secondary metabolites from *Cissus quadrangularis* were elucidated by molecular docking approaches [51]. Bioactive compounds from endophytes are gaining importance to be explored as therapeutic agents for treatment of various diseases including cancer [52]. *In silico* analysis on these compounds helps us to understand the binding potential of compounds with the target enzyme. A detailed study on the compounds produced by endophytes of *Cissus quadrangularis* is required. From this study, it is found that the compounds docked had binding ability with Aurora B kinase.

4. CONCLUSION

This study indicates that the active compounds from the extracellular extracts of the endophytic bacteria hold lot of promise to develop as Aurora B kinase inhibitors, suggesting further work on the endophytic bacteria of *Cissus quadrangularis* and their compounds to be developed as potential drugs for cancer. The endophytic bacteria of *Cissus quadrangularis* holds great promise in developing drug candidates against many types of cancer including osteosarcoma. Natural molecules may be used in combination with conventional chemotherapy for increased treatment efficacy. Thus, the potential endophytic bacteria if extensively explored for their anticancer potential, may find its way into pharmaceuticals.

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Conflicts of interest

None declared

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