RESERACH ARTICLE

Exploring the Phytoconstituents from *Capparis zeylanica* L. Stem Extract: Phytochemical Analysis, Molecular Docking, and Anticancer Activity

Bhavana Jain[1*], Kishore Burade[2]

[1]*Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India.*
[2]*Department of Pharmacognosy and I/C Principal of Government College of Pharmacy, Karad, Maharashtra, India.*

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ABSTRACT

Ceylon caper (*Capparis zeylanica* Linn.), a climbing shrub found throughout the Indian subcontinent, has been utilized in traditional medicine in the direction of treating numerous ailments. The purpose of this study was to extract phytochemicals from *C. zeylanica* L.stems, evaluate the phytoconstituents and study the anticancer activity of the extract. Shade dried stems were collected, sieved and subjected to solvent extraction. Following standard phytochemical screening, We performed molecular docking studies to identify the most relevant pharmacological activities. *In-vitro* anticancer activity of ethanolic extract of *C. zeylanica* L. Stem was evaluated utilizing standard assay techniques. It indicates an interaction between extraction and EGFR kinases. And it found good IC_{50} values in enviro-enzymatic assay and inhibits phosphorylation of EGFR and its downstream signaling pathways in western blot assay.

Keywords: Anticancer, *Capparis zeylanica*, Anticancer activity, Molecular docking.

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INTRODUCTION

Cancer is a leading worldwide reason of demise, significantly affecting life expectancy.[1] Lung cancer types include NSCLCs, SCLCs, and neuroendocrine cancers, with NSCLCs comprising 80 to 85% of cases.[2] The EGFR is communal in tumors and shows a part in malignancy progression.[3] EGFR-TKIs are utilized to treat various cancers, but standard treatments like chemotherapy and radiotherapy can cause severe side effects and lead to drug resistance over time.[4]

Herbal medicines present a plausible alternative to the existing treatments. Several clinical researches have demonstrated a spectrum of anticancer actions of numerous herbal medicines. The phytochemicals and phyto-formulations have been evaluated as a standalone treatment or utilized as adjunct complementary treatments for cancer.[5] Along with anticancer activity, these herbal medicines exhibit beneficial effects on survival and immunomodulation, in addition to quality of life in malignancy patients.[6]

Capparis zeylanica Linn family Capparaceae, frequently recognized as Ceylon caper, is a climbing shrub found throughout the Indian subcontinent.[7] Parts of the plant and the entire plant have been utilized in traditional medicine in the direction of treating numerous ailments.[8] *C. zeylanica* Linn has been evaluated for its protective,[9] antidiarrheal,[10] analgesic, antipyretic,[11] antimicrobial,[12] immunostimulant,[13] properties. But nobody find out on anticancer activity. However, the combined data on the identification, characterization, evaluation, and molecular docking of phytoconstituents of *C. zeylanica* Linn are rare.

The study aimed to extract phytoconstituents from *C. zeylanica* L stems and to evaluate the phytochemicals and anticancer activity of the extract. Furthermore, we carried out the identification, characterization, evaluation, and molecular docking of a novel phytoconstituent form *C. zeylanica* L. stem extract.

MATERIALS AND METHODS

Materials

The plant specimen was collected in the month of June from the Solapur region of Maharashtra, India. The Botanical Survey of India, Pune, Maharashtra, verified the plant specimen as *C. zeylanica* L.(Ref No./No BSI/WRC/ Idn.Cer 2022.0308220025110

**Author for Correspondence: jigisha1619@gmail.com*

Methods

Extraction, isolation, and screening of phytoconstituents

The stems of the plant were dried in the shade, prepared from a course power. About 250 g of the resulting powder was successively extracted with pet ether (60–80%) and chloroform, then with ethanol in the soxhlet apparatus. The extract was tested for the presence of numerous phytoconstituents. We utilized the extraction method.[14]

Qualitative phytochemical screening

We carried out an initial phytochemical analysis to determine the chemical constituents present and used established tests from the literature for the screening process.[15-18]

In-vitro enzymatic action

The inhibitory action of ethanolic extract of *C. zeylanica* L. stem (EECAZ) on selective mutant EGFR L858R/T790M/C797S was tested *in-vitro* utilizing osimertinib as a standard. Assays were conducted at 30°C for 40 minutes with specific concentrations of tris, $MgCl_2$, BSA, DTT, ATP, kinase, and enzyme substrate. A compound in 10% DMSO was added to achieve 1% DMSO. The kinase-Glo Plus luminescence kit measured ATP levels, indicating kinase action. IC_{50} values were estimated utilizing GraphPad Prism 5.0.[19]

Western blot assay

In the direction of investigating the anticancer mechanism ethanolic extract of *C. zeylanica* L. stem (EECAZ), an assay was conducted on HCC827 cells treated with 0.01, 0.10, and 1.00 µM of the extract, and 1-µM synthesized analogs for 1-hour. Cells were treated with EGF immediately or after washing. Proteins from whole-cell lysates were extracted, parted by SDS–PAGE, transferred to PVDF membranes, and probed with EGFR and p-EGFR antibodies. Detection was done utilizing peroxidase-coupled secondary antibodies and an ECL-plus kit.[20]

Computational studies

Docking studies of EECAZ in contrast to EGFR-TK, were conducted utilizing Smina molecular docking software. Ligand structures were created in Marvin Sketch, transformed to 3D, and energy-minimized with Open Babel. The enzyme's X-ray crystal structure was obtained from the Protein Data Bank and prepared utilizing UCSF Chimera. Lower energy conformers of the ligands were docked into the enzyme's active site utilizing Smina's default scoring function. Results were visualized and saved with Maestro software.[21]

Molecular dynamics (MD) simulations were performed utilizing Desmond for EECAZ -WT-EGFR, ethanolic extract of *C. zeylanica* L. stem (EECAZ) -EGFRT790M, and EECAZ-EGFRL858R/T790M complexes. The complexes were solvated with SPC water, neutralized with ions, and minimized. Simulations included equilibration and production runs (1 ns and 100 ns), utilizing the OPLS_2005 force field. Temperature and pressure were set to 300 K and 1.01325 bars, correspondingly, with Coulomb interactions managed by Particle Mesh Ewald. Trajectories were recorded every 10 picoseconds, and metrics such as RMSD, RMSF, and ligand interactions were analyzed utilizing Desmond.[22-24]

RESULTS

Extraction, Isolation and Screening of Phytoconstituents

The ethanolic extract had a higher concentration of phytoconstituents compared with the other extracts (pet ether and chloroform). The ethanolic extract exhibited positive outcomes for the existence of alkaloids and flavonoids, as shown in Table 1. The extractive value of the dried ethanolic extract was found to be approximately 20%.

In-vitro Enzymatic Activity Assay

Osimertinib is a potent tyrosine kinase inhibitor that selectively inhibits mutant EGFR; however, the third mutation C797S causes resistance against osimertinib.[25-29] The IC_{50} values for *in-vitro* enzymatic inhibitory activity for EECAZ and osimertinib against EGFR - L858R/T790M/C797S were 146 ± 0.16 and 122 ± 0.12 nM, correspondingly. The results of *in-vitro* enzymatic inhibitory activity showed that ethanolic extract of *C. zeylanica* L. stem extract had an upright inhibitory effect on the osimertinib-resistant triple mutant EGFR -L858R/T790M/C797S.

Western Blot Assay

Phosphorylation of the tyrosine kinases, such as EGFR triggers the phosphorylation and activation of the downstream signaling pathway. We assessed the phosphorylation levels of EGFR and AKT after treatment with EECAZ utilizing western blot assay. The blots observed on the gel are shown in Figure 1. Maximum effect was detected at the concentration of 1-µM in both EECAZ and osimertinib. Results of the assay show that EECAZ inhibits the phosphorylation of EGFR and its downstream signaling pathways.

Computational Studies

The RMSD values for ligands from PDB 4I23, 2JIU, and 5D41 were 0.65, 1.38, and 0.68, correspondingly. Docking results showed EECAZ binds effectively to together the ATP binding place and allosteric place of EGFR kinase, with binding affinities of -6.98, -6.74, and -5.81 for WT-EGFR, EGFR-T790M, and EGFR-L858R/T790M/C797S, correspondingly.

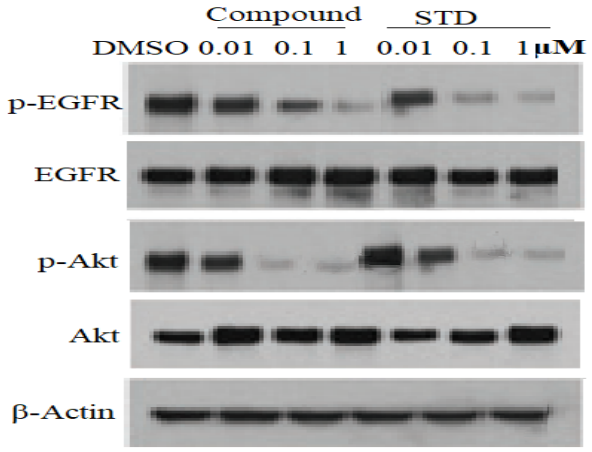
Figure 2 shows the RMSD evaluation of all three systems over 100 ns of MD simulations. The RMSD values for the ethanolic extract (CAZEE)-WT-EGFR and EECAZ-EGFRT790M complexes indicated stability throughout the simulations. However, the RMSD for the EECAZ-EGFRL858R/T790M/C797S complex increased over time, with the ligand-protein RMSD fluctuating around 3.6 Å between 15 and 30 ns before stabilizing between 0.8 and 2.0Å for the remainder of the simulation.

Additionally, the RMSF values for Cα atoms in amino acid residues showed lower atomic fluctuations for all three systems, except in the loop regions of the EGFR-T790M and EGFR-L858R/T790M/C797S enzymes. This directs minimal conformational changes and suggests that each amino acid remained stable in all three systems Figure 3. Thus, the MD

Table 1: Screening of the EECAZ utilizing numerous phytochemical tests*

Phytochemical test	*Procedure/reagents utilized*	*Observation*
1 Test for alkaloids (Extracts were dissolved in dil hydrochloric acid and filtered)	1. Mayer's test Resulting solvent tested with Mayer's reagent (potassium mercuric iodide)	Formation of a yellow colored precipitate
	2. Wagner's test: Resulting solvent tested with Wagner's reagent (iodine in potassium iodide)	Formation of brown/reddish precipitate
	3. Dragendorff's test: Resulting solvent tested withpotassium bismuth iodide	Formation ofa red-colored precipitate
2.Test for Flavonoids Compound	4. Hager's test: Resulting solvent tested with picric acid 1.Shinoda Test: - Extract was tested with Zn powder and few drops of conc HCL to 2ml of solution	Formation of deep red to magenta color
	1. Ferric chloride test: Extract was tested with a few drops of ferric chloride solution	Development of a dark blue color
	2. Lead acetic acid test Extract was tested with 2–3 drops of 5% lead acetic acid solution	Development of a white precipitate

*Only positive tests are reported her



STD, Standard drug (Osimertinib)

Figure 1: Inhibition of EGFR autophosphorylation in HCC827 cells.

simulation confirmed that EECAZ formed a stable complex with the EGFR enzyme with minimal structural alterations.

The docking study predictions were confirmed by the MD simulation of the EECAZ-WT-EGFR complex, which showed that EECAZ formed hydrogen bonds with the Met 793 residue for 85% of the simulation time and through a water bridge for 23%. The simulation also revealed interactions with Arg 841 *via* hydrogen bonds and with Asp 855 through pi-pi interactions within the WT-EGFR active site Figures 4 and 5. Similarly, the EECAZ-EGFRT790M complex analysis confirmed hydrogen

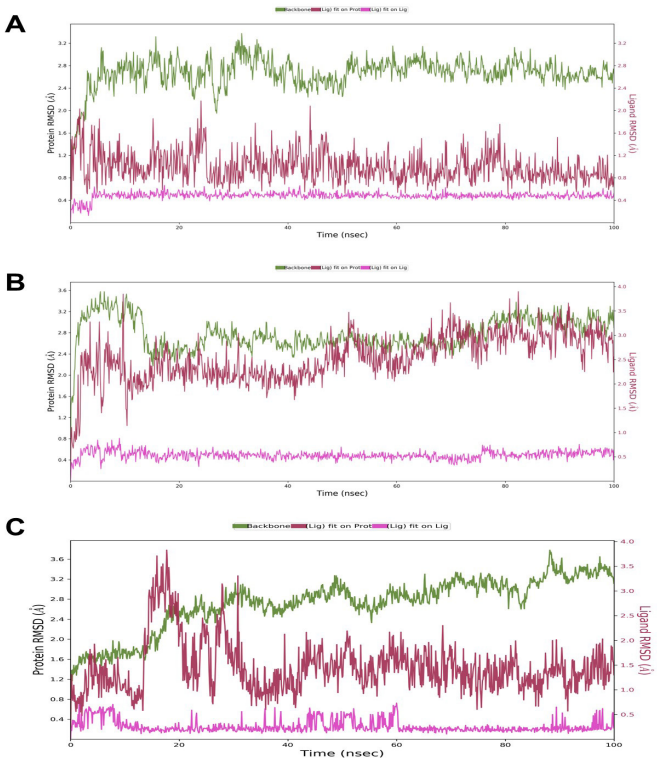


Figure 2: RMSD of all three systems (a) EECAZ-WT-EGFR complex (b) EECAZ- EGFR-T790M complex and (c) EECAZ- EGFR-L858R/T790M/C797S complex over 100 ns of MD simulations. EGFR; MD is molecular dynamics; RMSD; WT is wild-type

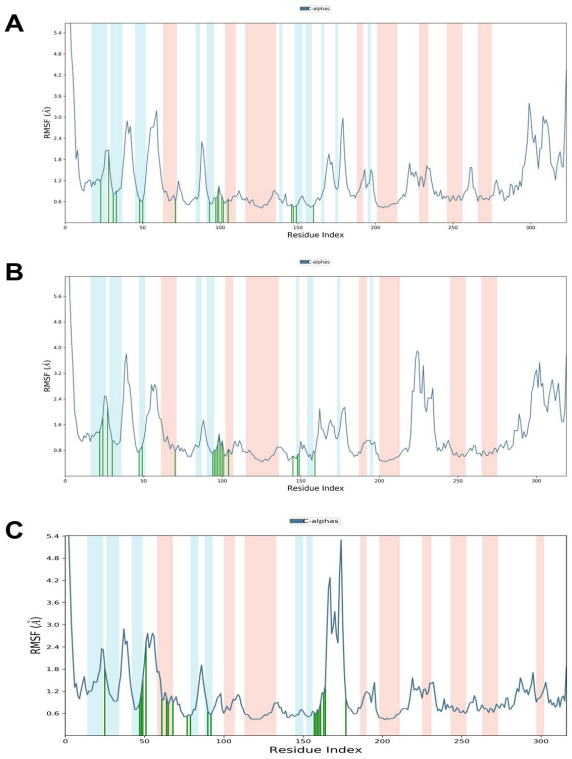


Figure 3: RMSF of protein Cα atoms of protein of all three systems (a) EECAZ-WT-EGFR complex (b) EECAZ- EGFRT-790M complex and (c) EECAZ- EGFR-L858R/T790M/C797S complex over 100 ns MD simulations. EGFR; MD: molecular dynamics; RMSF; WT: wild-type

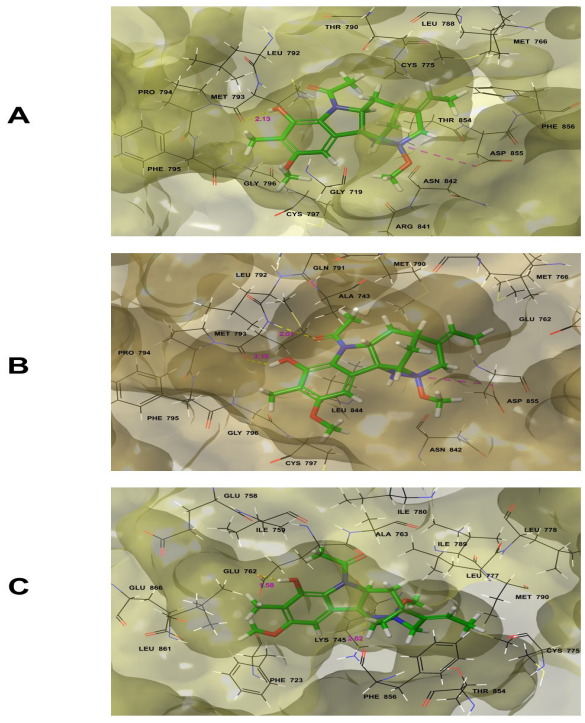


Figure 4: Docking pose of EECAZ in the active place of (a) WT-EGFR enzyme (b) EGFR-T790M enzyme and (c) in the allosteric site of EGFR-L858R/T790M/C797S enzyme. Yellow dotted line directs H-bonds and pink dotted line directs pi-pi interactions. EGFR; MD; WT, wild-type

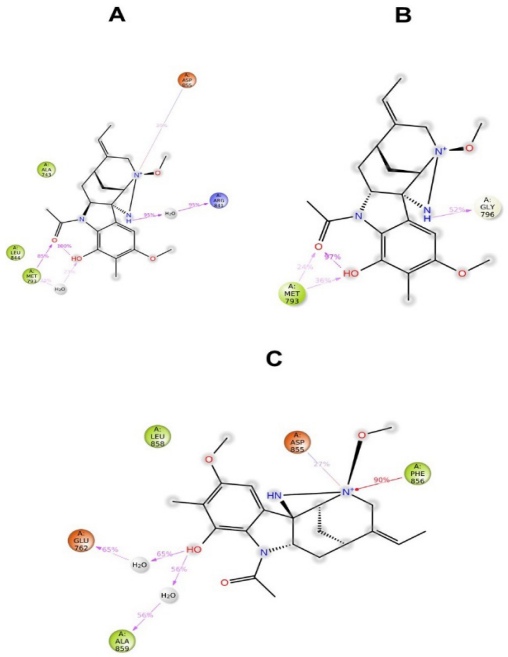


Figure 5: A schematic of the interaction of EECAZ with active site amino acid residues during 100 ns MD simulations time period (a) WT-EGFR enzyme (b) EGFR-T790M enzyme and (c) EGFR-L858R/T790M/C797S enzyme. EGFR; MD; WT, wild-type

bonds with Met 793, and although the docking pose predicted an additional hydrogen bond with Leu 792, this was less frequent in the MD simulation. Instead, the simulation showed a hydrogen bond between the EECAZ and Gly 796. For the compound-EGFRL858R/T790M/C797S complex, the MD simulation identified stable hydrogen bonds with Arg 762 and Ala 859 for over 50% of the simulation time, and also a pi-pi interaction with Phe 856 Figures 4 and 5.

DİSCUSSİON

The study explored the pharmacological properties of ethanolic extract of EECAZ particularly its potential as an EGFR inhibitor and its impact on cancer cells. The ethanolic extract showed a higher concentration of phytoconstituents than pet ether and chloroform extracts, with positive results for alkaloids and flavonoids. *In-vitro* assays demonstrated that the EECAZ effectively inhibits the osimertinib-resistant EGFR triple mutant (EGFR-L858R/T790M/C797S), with an IC_{50} of 146 ± 0.16 nM, compared to osimertinib's 122 ± 0.12 nM. Western blot analysis confirmed that the extract blocks EGFR phosphorylation and its downstream signaling pathways. Computational studies revealed that EECAZ, the active compound, forms stable complexes with EGFR variants, effectively binding to ATP and allosteric sites, with interactions involving hydrogen bonds and pi-pi interactions. Overall, these findings suggest that EECAZ has significant potential as an EGFR inhibitor and anticancer agent, with possible therapeutic applications for addressing drug-resistant mutations in cancer treatment.

CONCLUSION

In conclusion, we isolated and identified a novel compound, EECAZ, from the stems of *C. zeylanica*. Molecular docking was executed in the direction of investigating the inhibitory activity of EECAZ against L858R/T790M/C797S mutated EGFR kinases. These observations from our study show that EECAZ is a naturally derived selective EGFR-L858R/T790M kinase inhibitor. The promising results suggest that the anticancer properties of this compound should be studied further in preclinical and clinical studies.

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