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# EVALUATION COLORECTAL CANCER ACTIVITY USING *ALANGIUM SALVIIFOLIUM* PHYTOCHEMICALS THOUGH *INSILICO* APPROACH

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**ABSTRACT**:
The use of natural products to reduce the negative effects of marketed cancer medications is becoming more popular. This investigation utilized the components of *Alangium salviifolium*. Plants have alkaloids with anticancer effects, specifically beta-carboline harmaline and deoxytubulosine. CADD, a computational-based design technique, was employed to comprehend the anticancer properties of the alkaloids. The anticancer properties of the compounds were evaluated using the NPACT database, and the AutoDock program was used to test how well they bind to six different receptors. The results were displayed in Discovery Studio. These outcomes were assessed using Paclitaxel, a commonly marketed medication. Zinc oxide was added to the compounds to provide further advantages. Following adjustments, Discovery Studio displayed the findings of the molecular docking analysis conducted using the PyRx program. Beta-carboline harmaline must be validated in vitro for the pharmacokinetics study utilizing the pkCSM database.
***KEYWORDS***: Cancer, Colorectal, *Alangium salviifolium,* anticancer, Bioinformatics, AutoDock.

## INTRODUCTION:

Colorectal cancer (CRC) remains one of the most prevalent and lethal malignancies globally, with increasing incidence in both developed and developing nations (1). Despite advances in screening and treatment, challenges persist due to drug resistance, side effects, and high recurrence rates (2). Therefore, identifying novel, effective, and safer therapeutic agents is critical for improving CRC management. Natural products derived from medicinal plants have historically played a crucial role in drug discovery, especially in oncology (3). *Alangium salviifolium*, a traditional medicinal plant belonging to the family Cornaceae, is well known in Ayurveda and folk medicine for its diverse pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial effects (4-6). The plant *A. salviifolium* contains phytochemicals like alkaloids, flavonoids, phenolics, and glycosides, which are thought to have strong effects that could be used to fight different types of cancer (5). *In silico* approaches such as molecular docking and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction have emerged as efficient, cost-effective strategies to evaluate the drug-likeness and biological activity of plant-derived compounds before in vitro or in vivo testing (7-10). These computer-based methods help find possible connections between phytochemicals and important molecules related to colorectal cancer development, like COX-2, EGFR, KRAS, and β-catenin (11-14). This study aims to assess the potential of certain plant chemicals from *Alangium salviifolium* to fight colorectal cancer using computer-based methods. Using molecular docking and pharmacokinetic

prediction, we aim to find promising bioactive candidates that could help in developing new treatments for colorectal cancer (15).

## MATERIAL AND METHODS:

The standard medication and its active ingredients were obtained from the DrugBank and PubChem databases. The anticancer characteristics of active constituents have been predicted using the NPACT (Naturally Occurring Plant-Based Anticancerous Compound-Activity-Target) database. Based on the features of the test and standard drugs, the target molecule was found using the RCSB Protein Data Bank (PDB) for various target molecules (16,17).

**Prediction of binding sites of a target molecule:**

The area where ligands attach during a biological reaction is known as the target molecule's binding site. The location of the binding site in a target molecule was predicted using the CASTp (Computed Atlas of Surface Topography of Proteins) database (18).

Binding activity study of the target molecule and active constituents:

Molecular docking for the binding affinity study was carried out using AutoDock 4 software (MGL tools), which generates binding models along with their binding affinity and RMSD value, between the target molecule and the active ingredients of the plant and the control medication, paclitaxel (19).

**Modification of compounds:**

The Molinspiration cheminformatics database, which offers the bioactivity of the compounds from which it may be inferred whether the chemical was formed correctly or incorrectly, was utilized to improve the research drug (20). This database constructs the compound using the canonical GRINS format. The addition of the zinc oxide molecule changed the compounds. The binding affinity and RSMD values for the molecular docking of the changed compounds were obtained using PyRx software (21-24).

**Pharmacokinetics prediction of the compound:**

To ascertain the compounds absorption, distribution, metabolism, excretion, and toxicity, pharmacokinetic prediction was used. The forecast was made using the pkCSM tool database (25-27).

## RESULTS AND DISCUSSION

**Retrieval of the active constituents and the standard drug**

According to S. Kim et al., PubChem is an open database of chemicals and information about their biological activity. As a result, it was employed to retrieve common medications and active ingredients. Beta-carboline harmaline and deoxytubulosine were the study's active ingredients. For this trial, paclitaxel was regarded as a conventional medication. PubChem was used to acquire the structure and the canonical SMILES. Table 1 lists the study's structure, canonical smiles, molecular weight, molecular formula, and PubChem ID. S. Bundela et al. identified possible chemicals for the treatment of oral cancer using PubChem. According to D. Wishart *et al.,* DrugBank is an online database that offers details about medications that are available on the market and is based on experiments. Paclitaxel was the standard medication utilized in this investigation. CRC is treated with paclitaxel. The bark of the Pacific Yew tree, which includes endophytic fungus that produces paclitaxel, is the source of this mitotic inhibitor. Figure 1 depicts the structure of paclitaxel.

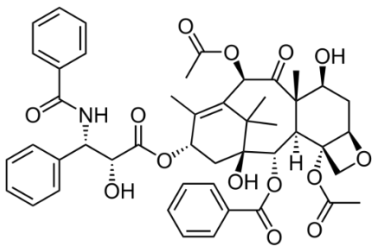
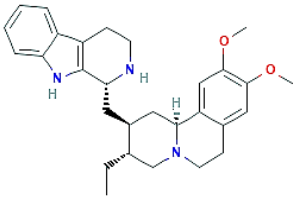
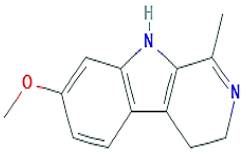



Figure 1: Structure of Paclitaxel

Table 1: Details of active constituents

| Name of compounds | Deoxytubulosine | Beta-carboline Harmaline |
|---|---|---|

| PubChem ID | 165003 | 3564 |
|---|---|---|
| Molecular Formula | $C_{29}H_{37}N_3O_2$ | $C_{13}H_{14}N_2O$ |
| Molecular Weight | 459.6 g/mol | 214.26 g/mol |
| Structure |  |  |

Anticancer properties prediction of the compounds:

The NPACT tool, as provided by M. Manga *et al.*, was utilized in this work to make predictions. In this case, 0.05 µg/mL of deoxytubulosine is the dosage that produces a 50% impact. Beta-carboline harmaline and the common medication paclitaxel have IC50 values of 34±12 µM and >10 µg/ml, respectively. The inhibitory concentration needed to stop biological or metabolic activity is known as the IC50. Administering the medication paclitaxel, which has an IC50 value of 34±12 µM, will prevent 50% of cancer from progressing. Similarly, beta-carboline harmaline, whose IC50 value is greater than 10 µg/ml, will prevent the biological activity of the cell that causes cancer to spread.

**Retrieval of the target molecule**

The receptors in the cells where the ligand attaches and initiates the signaling cascade are known as target molecules. The protein found in the cell that contributes to the progression of the disease was the target molecule in this investigation. Drugs can target this protein to generate a therapeutic impact. The PDB database was used to retrieve the target molecule. The target molecules used are TGF, EGFR, IGFR, estrogen receptor, integrin receptor, and tubulin receptor, each identified by their specific PDB IDs: 1TGJ, 3VJO, 5FXR, 1YYE, IU8C, and 6QVE. Figure 2 describes the target molecules' three-dimensional structure.

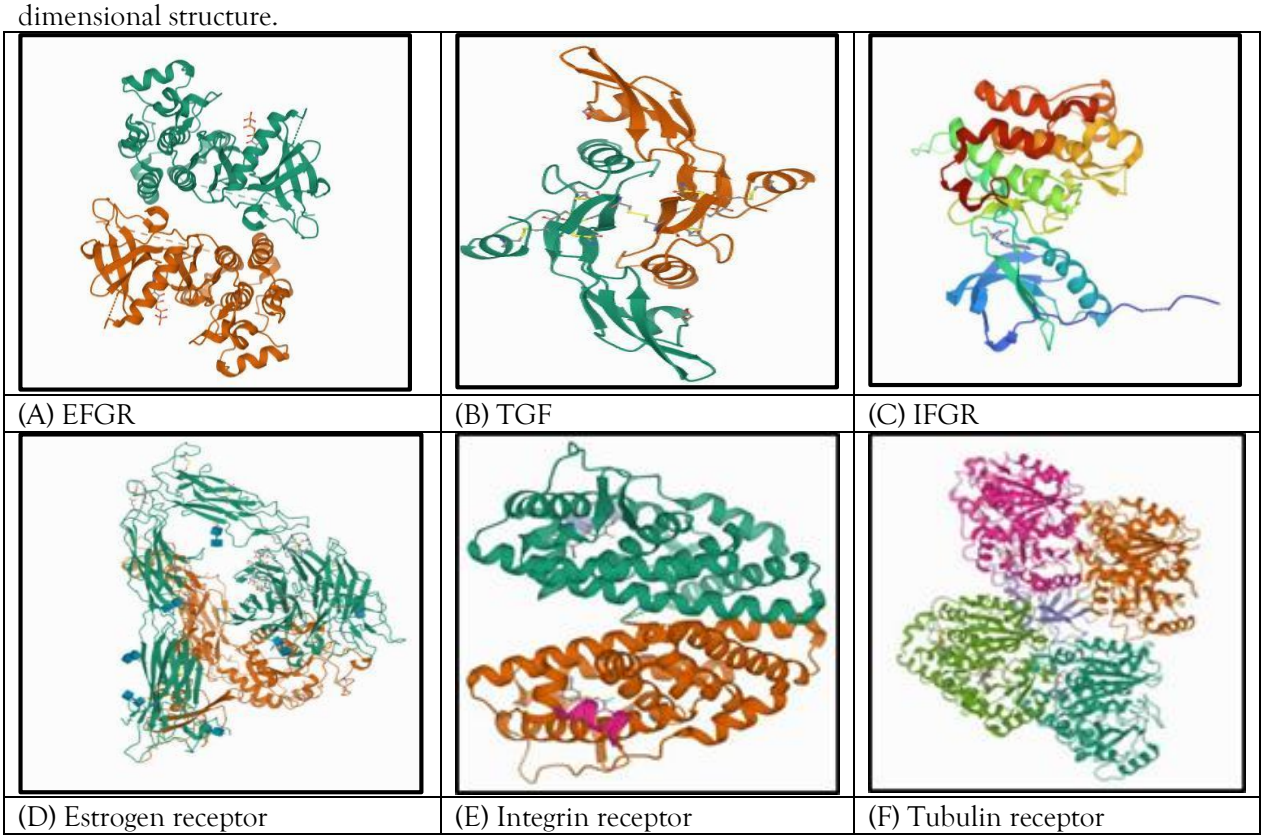



Figure 2: Structure of the target molecule

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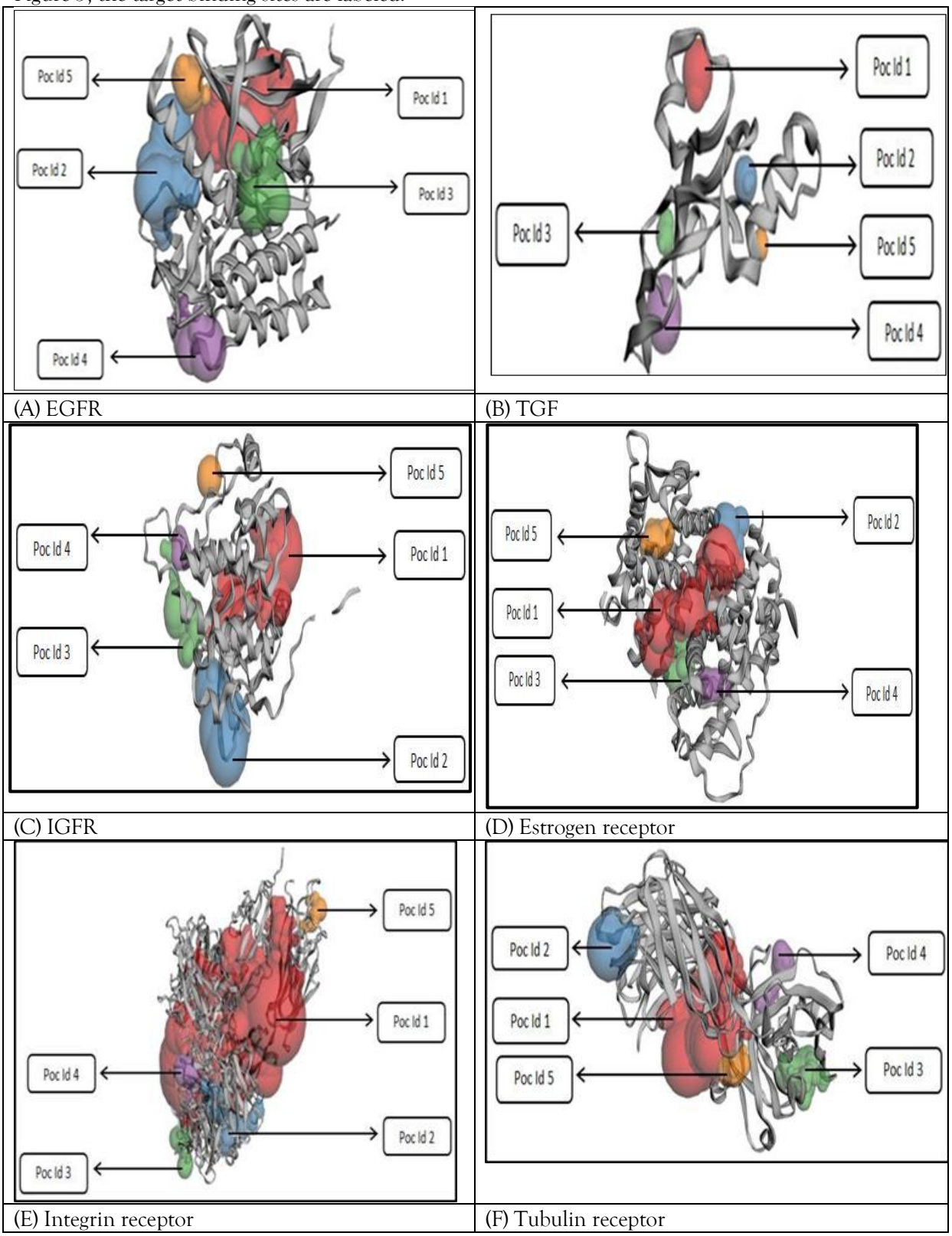
**Prediction of binding sites of the target molecule**

The area where the ligand binds during the biological reaction is known as the protein's binding site. The biological reaction engages a protein with a large number of binding sites. The CASTp server was used to predict a protein's binding site. We selected five out of the numerous binding sites we found. As mentioned, the ligand binds to 85% of the larger binding site, which has a greater size and volume. In Figure 3, the target binding sites are labeled.




(A) EGFR (B) TGF

(C) IGFR (D) Estrogen receptor

(E) Integrin receptor (F) Tubulin receptor

Figure 3: Binding site of target molecule

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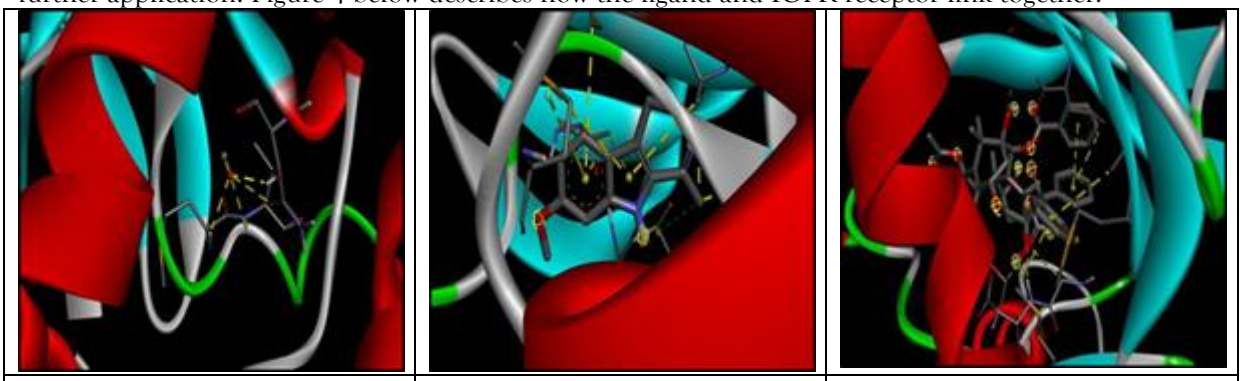
**Binding affinity study of target molecule and ligand**

The binding affinity refers to the strength of the reversible binding relationship between the ligand and target molecules. How exactly a ligand binds to its target molecule is determined by its binding affinity. Molecular docking is used to ascertain the ligand and target molecule's binding affinities. One technique for predicting how a target molecule and a ligand molecule would fit together is called molecular docking. A common molecular modeling technique for predicting how a protein will interact with tiny molecules is docking. Therefore, we utilize a wide variety of molecular docking methods to predict the binding affinity. This study employs the AutoDock technology for molecular docking. Table 2 describes the ligand molecule's binding affinity with the receptor.

Table 2: Binding affinity of the target molecule with ligand

| Name of the target molecule | Deoxytubulosine (kcal/mol) | Beta-carboline harmaline (kcal/mol) | Paclitaxel (kcal/mol) |
|---|---|---|---|
| EGFR | -1.6 | -8.3 | -6.4 |
| TGF | -1.5 | -5.6 | -5.7 |
| IGFR | -1.8 | -7.7 | -8.2 |
| Estrogen receptor | -1.7 | -6.0 | -6.7 |
| Integrin receptor | -1.8 | -5.7 | -6.7 |
| Tubulin Receptor | -1.3 | -5.9 | -7.0 |

It is possible to infer from the table that the binding affinities of the drugs paclitaxel and deoxytubulosine differed greatly. However, the medications paclitaxel and beta-carboline harmaline did not have the same binding affinity. Beta-carboline harmaline can now be utilized as a lead compound to create a medication that can be used to treat colorectal cancer. Table 2 shows that the IGFR's binding affinity with the ligand molecules was highest for deoxytubulosine (-1.8 kcal/mol), beta-carboline harmaline (-7.7 kcal/mol), and paclitaxel (-8.2 kcal/mol). Therefore, we selected the IGFR receptor for further application. Figure 4 below describes how the ligand and IGFR receptor link together.



*(A) IGFR with deoxytubulosine* | *(B) IGFR with Beta-carboline harmaline* | *(C) IGFR with Paclitaxel*

Figure 4: Binding between IGFR and the compounds

**Modification of the compounds using bioinformatics tool**

We modified the chemical to increase its activity. If the activity rises, we can use the chemical as a lead compound to make the medication. In this investigation, we added zinc oxide groups to the molecules. We modified the structure using the Molinspiration tool. We created the structure by inserting the canonical graphs into the Molinspiration tool. Table 3 outlines the properties of the modified compounds obtained through the Molinspiration tool.

Table 3: Properties of the modified compounds

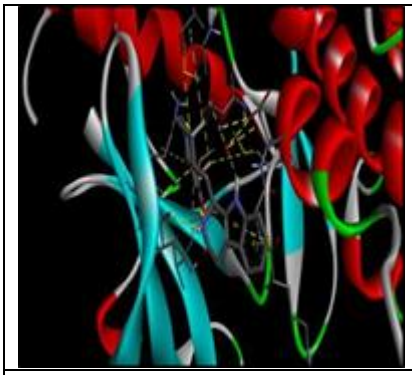
| Name of the compound | Deoxytubulosine + ZnO | Beta-carboline harmaline + ZnO | Paclitaxel +ZnO |
|---|---|---|---|
| MilogP | 4.09 | 1.64 | 3.67 |
| TPSA | 66.59 | 54.64 | 238.38 |

| | | | |
|---|---|---|---|
| natoms | 36 | 18 | 64 |
| Molecular weight | 540.01 | 294.65 | 934.30 |
| nOH | 6 | 4 | 16 |
| nOHNH | 2 | 1 | 4 |
| nrotb | 7 | 3 | 16 |
| Volume | 485.54 | 240.22 | 795.67 |

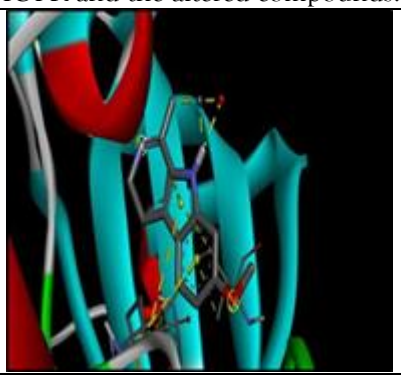
The drug's permeability to the target tissue is assessed using the logP value. The medication is lipophilic if the logP value is higher than 1. Lipinski's rule suggests that the logP value should be greater than 5. Table 3 shows that all three compounds changed by the addition of ZnO had logP values greater than 1. It indicates that every molecule is lipophilic. Molecular Polar Surface Area, or TPSA, is measured for blood-brain barrier, $CACO_2$ permeability, bioavailability, and absorption. A compound is considered to have poor characteristics if its TPSA is larger than 140 Å. For optimal results, the TPSA should be less than 90 Å. The values of nOH (hydrogen bond donor) and nOHNH (hydrogen bond acceptor) ought to exceed 5 and 10. The rotatable bonds found in the compounds are denoted by nrotb. We examine this characteristic to determine the medicine's oral acceptability and to evaluate the molecule's flexibility. Since all of the chemicals are natural, they are not subject to Lipinski's rule. The compounds are also approved for usage in the future even if they do not adhere to Lipinski's criterion.

**Molecular docking of the target molecule and the modified compounds**

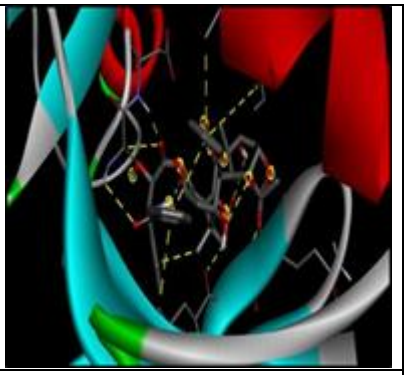
To ascertain the binding affinity and RMSD of the target molecule and the ligand, molecular docking was used. PyRx was used for the molecular docking in this investigation, and the Discovery Studio was used to show the outcome. The ligand molecule used in the molecular docking process is the modified compound created by adding ZnO to the original chemical—the receptor molecule IGFR. This compound demonstrated the highest binding affinity with the original compounds. Figure 5 below details the interaction between the IGFR and the altered compounds.



*A. IGFR with deoxytubulosine*



*B. IGFR with Beta-carboline harmaline*



*C. IGFR with Paclitaxel*

Figure 5: Binding between IGFR and the modified compounds

IGFR binds more strongly to modified deoxytubulosine at -9.3 kcal/mol, to modified beta-carboline harmaline at -7.7 kcal/mol, and to paclitaxel at -6.5 kcal/mol. Table 2 concludes that the molecule's binding affinity has increased with the addition of ZnO.

**Pharmacokinetics prediction of the compounds:**

To forecast toxicity, excretion, metabolism, distribution, and absorption, the compound's pharmacokinetic characteristics were examined. The ADMET describes the compound's pharmacological action as a medication. The ADMET forecast was made using the pkCSM tool. The database that uses graph-based signatures to predict the ADMET is called pkCSM. When the chemicals are sold, ADMET also helps lower their danger. The term "absorption" refers to the process by which the body absorbs a medicine after administration. The term "distribution" describes how a drug enters and exits blood and tissues as well as its proportion in the blood. Drug metabolism is defined as the biotransformation of the pharmacological ingredients to facilitate their easy removal from the body. The removal of a medicine in the form of a metabolite is known as excretion. A number of pathways,

including bile, sweat, tears, and urine, can eliminate the medicine. The term "toxicity" describes how toxic or detrimental a medicine is to the body when used.

Table 4: Characteristics of the ADMET properties

| Properties | Characteristics | D | B | P | D1 | B1 | P1 |
|---|---|---|---|---|---|---|---|
| Absorption | $H_2O$ solubility | -4.057 | -3.123 | -3.158 | -3.949 | -3.386 | -3.08 |
| | $CaCO_2$ permeability | 0.883 | 1.621 | 0.623 | 0.734 | 1.294 | 0.551 |
| | Intestinal absorption | 91.208 | 93.622 | 100 | 90.441 | 93.69 | 100 |
| | P glycoprotein substrate | Yes | Yes | Yes | Yes | Yes | Yes |
| Distribution | VDss | 2.005 | 0.264 | 1.458 | 1.759 | 0.104 | 1.404 |
| | Unbound fraction | 0.18 | 0.267 | 0 | 0.196 | 0.253 | 0.013 |
| Metabolism | CYP2D6 | Yes | Yes | No | Yes | Yes | No |
| | CYP3A4 | Yes | No | Yes | Yes | Yes | No |
| | CYP1A2 | Yes | Yes | No | Yes | Yes | No |
| | CYPC19 | No | No | No | Yes | No | No |
| | CYP2D6 | No | No | No | No | No | No |
| | CYP2C9 | Yes | No | No | Yes | No | No |
| | CYP3A4 | No | No | Yes | No | No | Yes |
| Excretion | Total clearance | 1.057 | 0.576 | -0.36 | 2.293 | 1.584 | -0.838 |
| | Renal OCT2 substrate | Yes | Yes | No | Yes | No | No |
| Toxicity | AMES | No | No | No | No | No | No |
| | Maximum tolerated dose | -0.154 | -0.179 | 0.199 | -0.161 | -0.16 | 0.244 |
| | hERG I | No | No | No | No | No | No |
| | hERG II | Yes | No | Yes | Yes | No | Yes |
| | Oral rat acute toxicity | 2.622 | 2.452 | 2.776 | 2.495 | 2.448 | 2.76 |
| | Oral rat chronic toxicity | 1.266 | 1.699 | 3.393 | 1.457 | 1.333 | 3.382 |
| | Hepatotoxicity | Yes | No | Yes | No | No | Yes |
| | T. pyriformis | 0.327 | 1.34 | 0.285 | 0.3 | 1.664 | 0.285 |
| | Minnow toxicity | -0.854 | 0.529 | 2.988 | -1.159 | 0.099 | 2.807 |

(*D- Deoxytubulosine, B- Beta-carboline harmaline, P- Paclitaxel, D1- Deoxytubulosine with ZnO, B1- Beta-carboline harmaline with ZnO, P1- Paclitaxel with ZnO)

The main focus of ADMET characteristics is on toxicity, distribution, and absorption. According to Table 4, modified beta-carboline harmaline and beta-carboline harmaline showed better absorption and distribution results. Additionally, it has been shown that it is less harmful than the common medication Paclitaxel.

**CONCLUSION**:

Some of the plant chemicals that have anticancer effects are currently being employed. The plant A. salviifolium, which contains alkaloids thought to have anticancer effects, was used in this investigation.

Beta-carboline harmaline and deoxytubulosine are the alkaloids. The NPACT database was used to forecast the compounds' anticancer activity, and when compared to the common medication paclitaxel, beta-carboline harmaline should have greater activity than deoxytubulosine. The study found that beta-carboline harmaline binds better to the IGFR receptor with a score of -7.7 kcal/mol compared to deoxytubulosine at -1.8 kcal/mol and paclitaxel at -8.2 kcal/mol. Despite the ZnO group being added to boost activity, these results showed that beta-carboline harmaline is superior. Since ZnO also possesses anticancer properties and may increase the compounds' activity, this change was made. Based on the compound's ADMET characteristics, beta-carboline harmaline was more effective and less harmful than deoxytubulosine and the common drug paclitaxel when adjusted with ZnO. It is determined that beta-carboline harmaline, a molecule with reduced toxicity that may result in fewer adverse effects, can be used further for in vitro testing of the medication.

**DECLARATIONS**:

Consent for publication:

All the authors approved the manuscript for publication.

Competing interests:

All authors declare no competing interests.

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