RESEARCH ARTICLE

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Identification of Novel Potential Herbal Drug Targets against Beta-Catenin in the Treatment of Oral Squamous Cell Carcinoma

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Abstract

Objective: The study aims to identify potential pharmacophore models for targeting beta-catenin, a crucial protein involved in the development of oral squamous cell carcinoma (OSCC), using a combination of herbal compounds and computational approaches. **Methods:** Five natural compounds namely Quercetin, Lycopene, Ovatodiolide, Karsil, and Delphinidin were selected based on their reported activity against beta-catenin. Ligand characteristics were analyzed using SwissADME to evaluate drug-likeness, lipophilicity (logP), and bioavailability. The three-dimensional structure of beta-catenin was retrieved from the Protein Data Bank (PDB). Pharmacophore modeling was performed using Pharmagist software, followed by molecular docking using Swissdock to assess binding interactions and energies. **Results:** Out of thousands of pharmacophore hits generated, 23 were selected based on drug-likeness properties. Molecular docking revealed that ZINC94512303, derived from the combination of the selected herbal compounds, exhibited the highest binding energy of -8.91 kcal/mol with beta-catenin, outperforming individual herbal compounds. This compound adhered to all drug-likeness rules and demonstrated optimal pharmacokinetic properties. **Conclusion:** The identified pharmacophore, ZINC94512303, shows promise as a therapeutic agent targeting beta-catenin in OSCC. The combination of computational drug design with herbal compounds offers a novel approach to enhance the efficacy of cancer treatment. Further pharmacokinetic and pharmacodynamic studies, along with in vitro and clinical evaluations, are recommended to validate the therapeutic potential of this compound.

Keywords: Oral cancer- drug design- herbal drugs- beta-catenin

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Introduction

Oral carcinogenesis is a complex, intricate process that involves accumulating genetic and molecular changes that cause cell growth, poor DNA repair, and abnormal cell death [1,2]. The Wnt signaling pathway controls various biological and physiological activities through canonical or non-canonical signaling pathways to activate various cellular signaling pathways. The Canonical signaling results in an increase in intra-nuclear accumulation of beta-catenin which is an E-cadherin-associated molecule on the cell membrane and participates in Ca2+-dependent cell-cell adhesion via E-cadherin/b-catenin complex [3,4]. Beta-catenin which is involved in various signaling events is crucial for controlling embryogenesis, stem cell renewal, and tissue maintenance [5]. The cadherins that beta-catenin binds control the structure's organization and activity [3,5]. Since beta-catenin has a function at the plasma membrane, their alterations can also influence the transcription of the E-cadherin gene, loss of cell-cell adhesion, and Wnt/-catenin signaling, and thus beta-catenin has a role in the development of numerous disorders in addition to tissue morphogenesis and cancer in various animals.

Wnt/-catenin signalling is abnormally activated in a variety of tumours, leading to neoplastic transformation, The membranous expression of beta-catenin has a restricting influence on cell proliferation and migration [1]. Therefore their abnormal expression in cancer, helps the disease develop, spread, and become resistant to treatment and the immune system [4]. Hence, direct targeting of beta-catenin could serve as an effective way to create anti-cancer drugs. Though various small molecules have been shown to directly bind the classical transcription factor beta-catenin which is a crucial component of the oncogenic Wnt signaling pathway, none of them has been

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shown to inhibit Wnt signaling by directly targeting beta-catenin, indicating that beta-catenin is still an untapped target for drug development [6].

Despite the fact that chemotherapy and radiotherapy are very effective cancer treatment options, they have serious adverse effects when used [7, 8, 9]. The progressive resistance of cancer cells to treatment is one of the key issues with cancer treatment. Therefore, one of the goals of immunopharmacological investigations to enhance the effectiveness of cancer treatment is to develop a novel strategy. Various potential areas of research are being conducted to find more biological and targeted treatments [10]. Natural products have been used to elucidate novel biological pathways and play a significant role in drug discovery [11, 12]. Recent research has uncovered a number of natural compounds strongly targeting b-catenin signaling [4].

Quercetin is found in different fruits, vegetables, seeds, nuts, green tea, and red wine. Studies have shown the anti-cancer properties of quercetin via various mechanisms including inhibition of enzymes that activate oncogenes, modulation of signal transduction pathways, and interaction with various proteins, enzymes, and receptors [13]. Another naturally occurring pigment called lycopene is produced by bacteria and photosynthetic plants. Lycopene has been proven to play a significant role in the prevention of cancer, owing to its antioxidant properties [14]. Ovatodiolide present in the medicinal plant Anisomeles indica (L.) Kuntze (Labiatae) is a bioactive ingredient with known anti-inflammatory activities. It has been shown to suppress tumorigenesis in OSCC by suppressing self-renewal activity [15]. Another non-toxic natural flavonoid silibinin (karsil) is present in milk thistle seed extracts and it has been shown to inhibit Wnt signaling and possess anti-cancer properties [16]. Delphinidin which is an anthocyanin inhibits pre-adipocyte differentiation through the activation of Wnt/beta-catenin signaling [17].

The selection of Quercetin, Lycopene, Ovatodiolide, Karsil, and Delphinidin for this study was based on their documented ability to modulate β-catenin signaling, a key pathway in oral carcinogenesis [13, 14, 15, 16,17]. Each herb has demonstrated potential anti-cancer properties, either by directly inhibiting β-catenin activity or through related mechanisms. Combining these herbs leverages their individual effects, potentially enhancing their therapeutic efficacy against OSCC and addressing the need for targeted, effective treatments. However, none of the previous studies have evaluated the cumulative effect of these herbs on beta-catenin protein. Computer-aided drug designing gives faster, more productive, and safer results compared to other drug designing methods by analyzing the interaction between the target and the ligands [18]. This study aims to explore the cumulative effects of five herbal molecules namely Quercetin, Lycopene, Ovatodiolide, Karsil, and Delphinidin on β-catenin using computational methods, providing a comprehensive approach to drug discovery for OSCC treatment.

Materials and Methods

Ligands identification

Five previously reported compounds targeting beta-catenin were selected namely Quercetin, Lycopene, Ovatodiolide, Karsil, and Delphinidin. The characteristics of the selected five natural compounds were analyzed using an online database ZINC15 which allows virtual screening by downloading the database subset (SMILE) of the chosen natural compounds respectivel [19]. After the ligand discovery, the format of the downloaded molecules was converted to mol2 format using openbabel software. Parameters such as Drug Likeness rules (Rule 1 - Lipinski, Rule 2- Ghose, Rule 3- Veber, Rule 4- Egan and Rule 5- Muegge) (Figure 1), logP value, and bioavailability were checked for the five chosen herbal ligands using SwissADME. SwissADME is an online tool used to predict the pharmacokinetics, drug-likeness, and medicinal chemistry properties of small molecules [20]. The software employs multiple rule-based filters, including Lipinski's Rule of Five, to assess the drug-likeness of compounds. LogP, or the partition coefficient, measures the lipophilicity of a compound by the ratio of its concentration in octanol to its concentration in water. A logP value between 1 and 3 is generally preferred, indicating an optimal balance for oral bioavailability, whereas values outside this range may suggest potential issues with absorption or distribution. Bioavailability refers to the fraction of an administered dose that reaches systemic circulation in its unchanged form, with values ranging from 0 to 1 (0% to 100%). High bioavailability is desirable, particularly for oral drugs, as it indicates efficient absorption and minimal first-pass metabolism. A bioavailability above 0.1 (10%) is typically considered adequate for therapeutic effectiveness, while values below this may indicate poor absorption or significant first-pass metabolism. By integrating these predictions, SwissADME allows for a rapid assessment of the potential success of small molecules as drug candidates.and their parameters were tabulated.

Target Protein structure from Protein data bank

The three-dimensional structure of beta-catenin, the target protein of interest in this study, was obtained from the Protein Data Bank (PDB). The PDB provides detailed atomic-level information on protein configurations, which is essential for accurate molecular modeling and docking [21]. The specific PDB entry for beta-catenin was selected based on its resolution and relevance to the study, ensuring that the structural data was suitable for evaluating interactions with the pharmacophores. This structural insight is essential for evaluating how different pharmacophores interact with beta-catenin and for identifying potential candidates that could effectively modulate its activity in the context of oral cancer treatment.

Pharmacophore detection using Pharmagist software

Pharmacophore is the spatial arrangement of features required for a molecule to interact with a specific target receptor. The pharmacophore detection is done using Pharmagist software by uploading the downloaded inputs

of five herbal compounds in mol2 format. Pharmagist is an online tool for pharmacophore detection based on the structural alignment of input molecules [22]. It uses ligand-based modeling to identify common pharmacophore features from a set of input molecules. The software's functional algorithm aligns the ligands and extracts the common spatial arrangement of key functional groups, allowing for the generation of potential pharmacophore models. In this study, the data of five herbs taken from Zinc15 software were uploaded in pharmagist in mol2 format to derive the pharmacophore hits obtained from combining all the five herbal ligands..

Selection of ligands using Swiss ADME

Pharmacophore virtual screening was done for the derived outputs by analyzing their drug likeness properties using SWISS ADME software [19] and verified for their violation in rules.

Molecular docking using Swissdock

The pharmacophores selected from the hits generated by the Pharmagist webserver were individually docked with the beta-catenin target protein using Swissdock, a web-based tool for molecular docking [23]. Swissdock utilizes the EADock DSS algorithm to predict the most favorable binding modes between the protein and ligands by exploring the potential binding sites and scoring the poses based on their binding energies. The docking process allowed for the identification of molecular interactions, such as hydrogen bonds, hydrophobic interactions, and electrostatic forces, between each pharmacophore and the target protein. The binding energies (measured in kcal/mol) for each pharmacophore were calculated and tabulated, providing insight into the strength of interaction and potential efficacy of each ligand. The pharmacophores with the most negative binding energies were considered the most stable and effective in binding to beta-catenin, suggesting their potential as therapeutic agents.

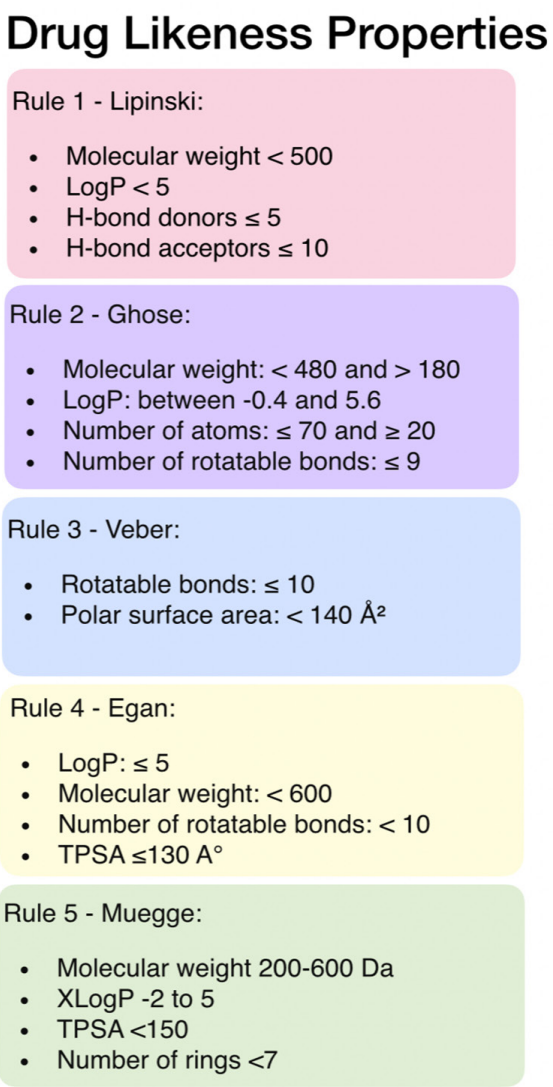


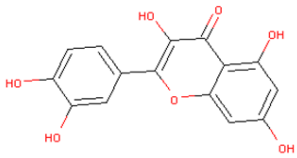
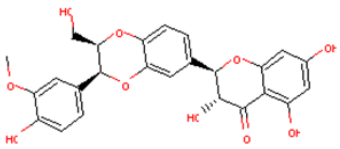
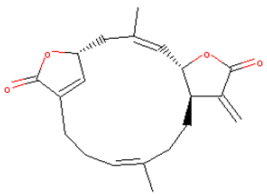
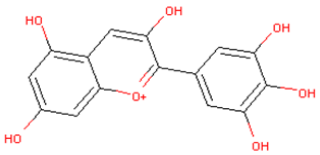
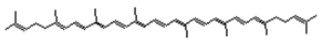
Figure 1. Showing Five Rules of Drug Likeness

Results

Ligand Identification and Characteristics

The structure of the five herbal ligands namely Quercetin, Lycopene, Ovatodiolide, Karsil, and Delphinidin were retrieved from the ZINC15 database [19] (Table 1). These compounds were analyzed using SwissADME to assess their drug-likeness and pharmacokinetic properties [20]. The parameters checked included Lipinski's Rule of Five, Ghose's rules, Veber's rules, Egan's rules, and Muegge's rules (Figure 1). Quercetin and Ovatodiolode obeyed all the rules of drug likeness. Lycopene obeyed

Table 1. Ligand Structures of Herbal Molecules Obtained from Zinc 15 Database

S.No	Name	Structure
1	Quercetin	
2	Karsil	
3	Ovatodiolide	
4	Delphinidin	
5	Lycopene	

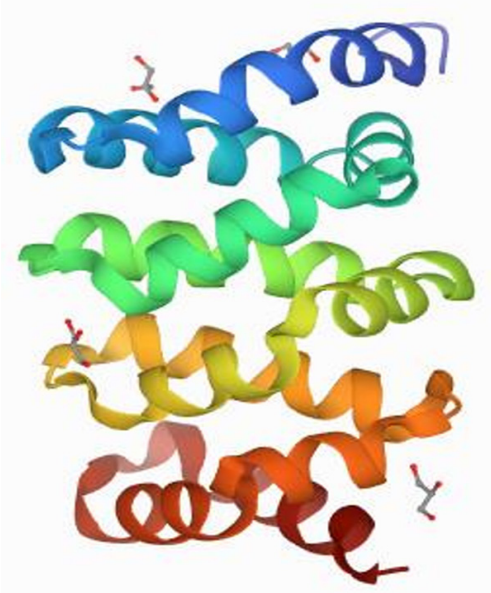


Figure 2. Protein (Beta Catenin) Structure Obtained from Protein Data Bank

Figure 3. Beta Catenin Protein Structures with Binding Sites (Structure Obtained after Docking)

four rules (rule 1, 2, 3, 5). Karsil obeyed one rule of drug-likeness (Rule 1). Delphinidin obeyed three rules of drug-likeness (Rule 1, 2 and 3). The calculated logP values and bioavailability for each compound is given in Table 2.

Target Protein Structure

The three-dimensional structure of beta-catenin was obtained from the Protein Data Bank (PDB) [21] (Figure 2). This structure provided detailed atomic-level information necessary for accurate molecular docking.

Pharmacophore Modeling

Pharmacophore modeling was performed using Pharmagist software [22]. The selected herbal ligands were submitted in mol2 format, resulting in the generation of thousands of pharmacophore hits. These pharmacophores represent the spatial arrangement of features required for the compounds to interact with beta-catenin.

Selection of Pharmacophores

From the thousands of pharmacophore hits derived, 23 pharmacophores were selected based on their drug-likeness properties evaluated using SwissADME [20]. These pharmacophores were chosen for further molecular docking.

Molecular Docking and Binding Energy Analysis

The 23 selected pharmacophores were individually docked with the beta-catenin target protein using Swissdock [23]. The EADock DSS algorithm was employed to predict favorable binding modes and calculate

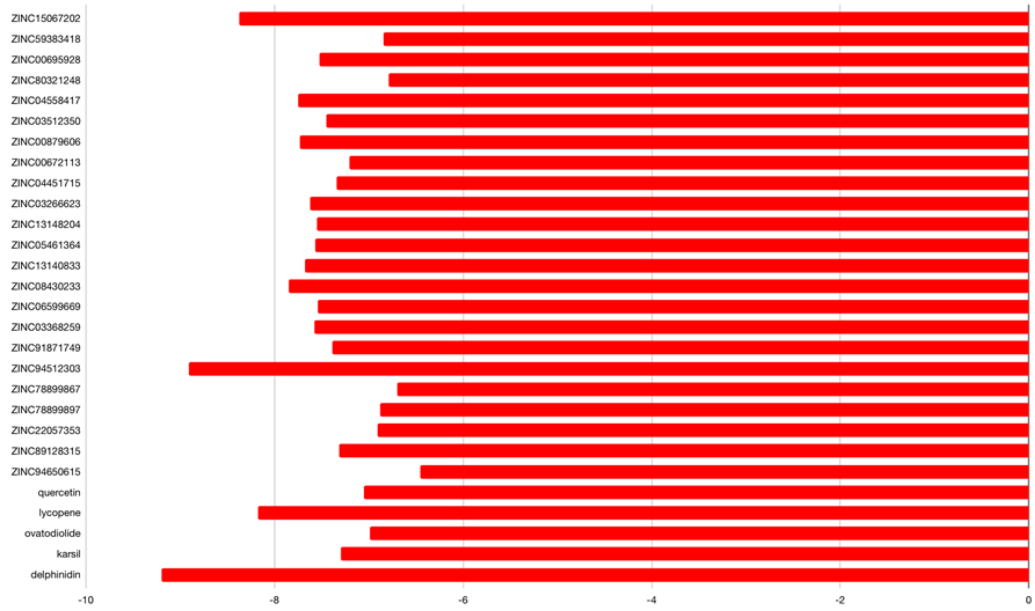


Figure 4. Comparison of Binding Energy of All the 23 Docked Molecules Comparable with the Binding Energies of Original 5 Herbal Ligands Selected for Docking

Table 2. Rules of Drug-Likeness, logP and Bioavailability of Original Five Herbal Ligands.

Herbal ligands	Rule1	Rule 2	Rule 3	Rule 4	Rule 5	logP	Bioavailability
Quercetin	Y	Y	Y	Y	Y	1.63	0.55
Lycopene	Y	Y	Y	N	Y	-	-
Ovatodiolide	Y	Y	Y	Y	Y	2.77	0.55
Karsil	Y	N	N	N	N	2.68	0.55
Delphinidin	Y	Y	Y	N	N	2.79	0.55

binding energies. The binding energies (measured in kcal/mol) for each pharmacophore were tabulated.

Optimal Pharmacophore Identification

The molecular docking revealed key interactions, such as hydrogen bonding and hydrophobic interactions, contributing to its high binding affinity. Among the pharmacophores evaluated, ZINC94512303 exhibited the highest binding energy of -8.91 kcal/mol (logP is 1.72, bioavailability score is 0.55) (Figure 3) (Table 3). This binding energy was notably higher than that of the individual herbal ligands (Figure 4). Additionally, ZINC94512303 adhered to all five drug-likeness rules (Lipinski, Ghose, Veber, Egan, and Muegge). It also had a logP value of 1.72 and a bioavailability of 0.55. The next ligand with highest binding energy was ZINC15067202, with a binding energy of -8.37 (logP is 3.57, bioavailability score is 0.55). A logP value of 1.57 of ZINC94512303 is typically more favorable for most drug applications due to its better balance of solubility and permeability. Consequently, ZINC94512303 is proposed as a potential therapeutic agent targeting beta-catenin (Table 4).

Discussion

Computer-assisted drug design and structural molecular biology are made easier by the use of molecular docking. Utilizing a scoring system, molecular docking can assess the binding affinity of a protein and its ligand as well as determine the intensity and complexity of their complicated interactions [24]. β-catenin signalling, a highly conserved pathway through evolution, regulates key cellular functions including proliferation, differentiation, migration, genetic stability, apoptosis, and stem cell renewal. The beta-catenin protein is linked to enhanced cell motility and proliferation and shows aberrant activation in OSCC [25]. Drugs targeting beta-catenin could act as a potent novel group of anti-cancer drugs.

Table 3. Rules of Drug-Likeness, logP and Bioavailability of the 23 Derived Molecules after Pharmacophore Modeling Using Pharmagist.

	Molecule ID of derived structures	Rule1	Rule 2	Rule 3	Rule 4	Rule 5	logP	Bioavailability
1	ZINC15067202	Y	Y	Y	Y	Y	3.57	0.55
2	ZINC59383418	Y	Y	Y	Y	Y	2.34	0.55
3	ZINC00695928	Y	Y	Y	Y	N	3.67	0.55
4	ZINC80321248	Y	Y	Y	Y	Y	3.55	0.55
5	ZINC04558417	Y	Y	Y	Y	N	3.62	0.55
6	ZINC03512350	Y	Y	Y	Y	Y	3.33	0.55
7	ZINC00879606	Y	Y	Y	Y	N	2.7	0.55
8	ZINC00672113	Y	Y	Y	Y	N	3.35	0.55
9	ZINC04451715	Y	Y	Y	Y	N	3.63	0.55
10	ZINC03266623	Y	Y	Y	Y	Y	3.34	0.55
11	ZINC13148204	Y	Y	Y	Y	N	3.6	0.55
12	ZINC05461364	Y	Y	Y	Y	Y	2.91	0.55
13	ZINC13140833	Y	Y	Y	N	Y	2.57	0.55
14	ZINC08430233	Y	Y	Y	Y	N	3.91	0.55
15	ZINC06599669	Y	Y	Y	Y	N	3.99	0.55
16	ZINC03368259	Y	Y	Y	Y	N	4.11	0.55
17	ZINC91871749	Y	Y	Y	Y	Y	2.95	0.55
18	ZINC94512303	Y	Y	Y	Y	Y	1.72	0.55
19	ZINC78899867	Y	Y	Y	Y	Y	2.54	0.55
20	ZINC78899897	Y	Y	Y	Y	Y	2.83	0.55
21	ZINC22057353	Y	Y	Y	Y	Y	1.73	0.55
22	ZINC89128315	Y	Y	Y	Y	Y	2.08	0.55
23	ZINC94650615	Y	Y	Y	Y	Y	2.91	0.55

Table 4. Binding Energies of All 23 Docked Molecules, Highlighting the Molecule with the Highest Binding Energy, and Comparing it with the Binding Energies of the Original 5 Herbal Ligands Selected for Docking

Herbal ligands	Docking score (Molecular binding energy)
Quercetin	-7.05
Lycopene	-8.17
Ovatodiolide	-6.99
Karsil	-7.30
Delphinidin	-9.20
Zinc IDs of newly derived structures	Docking score (Molecular binding energy)
ZINC94512303	-8.91
ZINC15067202	-8.37
ZINC59383418	-6.84
ZINC00695928	-7.52
ZINC80321248	-6.79
ZINC04558417	-7.75
ZINC03512350	-7.45
ZINC00879606	-7.73
ZINC00672113	-7.21
ZINC04451715	-7.34
ZINC03266623	-7.62
ZINC13148204	-7.55
ZINC05461364	-7.57
ZINC13140833	-7.68
ZINC08430233	-7.85
ZINC06599669	-7.54
ZINC03368259	-7.58
ZINC91871749	-7.39
ZINC78899867	-6.70
ZINC78899897	-6.88
ZINC22057353	-6.91
ZINC89128315	-7.31
ZINC94650615	-6.45

It has been observed that natural substances can increase the chemosensitivity of cancer cells by inhibiting the actions of proteins linked to drug resistance [26]. In the present study the five natural molecules namely, quercetin, lycopene, ovatodiolide, karsil, and delphinidin have been chosen from previous literature based on their individual property to target beta-catenin (Wnt Pathway) thus targeting the key regulating mechanism in the resistance of cancer stem cells to the anti-cancer drugs. A set of 23 molecules derived from the five herbal ligands were chosen and the binding energy of the new molecules was checked and compared to that of the five herbal ligands. It was found that among the 23 molecules, ZINC94512303 has the highest binding energy of -8.91, indicating a strong interaction with β-catenin. This molecule also adhered to all five drug-likeness rules, with a logP of 3.57 and a bioavailability score of 0.55, suggesting it possesses favorable pharmacokinetic properties and could be a promising candidate for further development as a therapeutic agent targeting β-catenin in OSCC.

Quercetin, one of the five natural ligands, is found to possess anti-cancer activity by playing an inhibitory role in angiogenesis [27, 28]. Thus, the increased binding energy of the novel drug may be because of the apoptosis-inducing, protein kinase C-inhibitory effect of quercetin. In another study by Yu-Kai su [3], the anticancer capability of another natural constituent Ovatodiolide has been demonstrated in glioblastoma and its adequacy in the therapy of glioblastoma as monotherapy [3]. This has been attributed to the inhibitory effects of activation of MAPK and Pi3K pathways which could be one of the contributory elements in increasing the activity of the novel drug against beta-catenin [29]. The anticancer activity of the novel drug could also be attributed to the presence of Silymarin which is shown to possess anti-proliferative and pro-apoptotic activity [30]. Another principle constituent, delphinidin has been reported to have potent anti-cancer activity by Yun et al and is attributed to their property of decreasing cell viability and arresting cell cycle in the G2/M phase [31]. This ligand itself is shown to possess the highest binding energy against beta-catenin but has violated two rules of drug-likeness.

The high binding affinity of ZINC94512303 can be attributed to its specific molecular interactions with the beta-catenin binding pocket. Key hydrogen bonds between ZINC94512303 and residues in the binding site, could have established strong interactions with polar amino acids. Hydrophobic interactions also play a role, with non-polar regions of the pharmacophore interacting with hydrophobic residues of the beta-catenin, further stabilizing the complex. Additionally, the structural conformation of ZINC94512303 would have allowed it to fit snugly within the binding site, optimizing both the hydrogen bonding and hydrophobic interactions. This conformation would have contributed to the higher binding energy of -8.91 kcal/mol compared to other ligands.

Therefore, this identified molecule ZINC94512303 may be useful in targeting beta-catenin and thus can play a major role in drug discovery for cancer. The high-throughput screening has made it possible to quickly examine a large number of prospective molecules, allowing for the selection of prototype ligands suitable for further development as therapeutic medicines [32]. The molecule identified in the study has the combined properties of all herbal drugs and may possess synergistic anticancer properties. Further, the new molecule is a combination of five herbal drugs and this would aid in overcoming the side effects associated with traditional cancer treatment. The integration of computational drug design with herbal medicine represents a significant advancement in therapeutic intervention, with far-reaching implications for the management of OSCC and other potentially malignant disorders.

In conclusion, the present study concluded that ZINC94512303 had better binding energy with the beta catenin protein among the 23 molecules and had properties superior to that of the five herbs chosen and has followed all the rules of drug likeness. Therefore can

be used in targeting beta catenin protein in the treatment of OSCC. Studies assessing pharmacokinetics as well as pharmacodynamics need to be done for the pharmaceutical development of the newly designed phytocompound. In future, Invitro studies and clinical studies are needed. The novelty of our findings lies in the formation of a molecular entity that embodies the combined pharmacological attributes of the constituent herbs, thereby heralding a new era in precision medicine for OSCC.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

None.

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