WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 13, Issue 18, 507-517. Research Article ISSN 2277–7105

INVESTIGATING AND COMPARING THE SUPPRESSIVE EFFECT OF A POLYHERBAL FORMULATION (PHF) BY USING *INVITRO* ANTICANCEROUS CELL LINES AND DOCKING STUDIES

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Article Received on
26 July 2024,
Revised on 15 August 2024,
Accepted on 04 Sept. 2024
DOI: 10.20959/wjpr202418-33725

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ABSTRACT

Herbal medicinal plants have been investigated for various pharmacological properties, yet many phytoconstituents remain unexplored. A polyherbal formulation (PHF) was developed by homogeneously mixing a specific proportion of *Justicia adhatoda, Glycyrhiza glabra, Withania somnifera, Cassia angustifolia, Zingiber officinale, Elletaria cardamomum, Terminalia chebula, Nigella sativa, Curcuma longa, Piper nigrum, Moringa oleifera, Cuminum cyminum, Phyllanthus emblica, Tribulus terrestris, Andrographis paniculata, Cinnamomum verum, Tinospora cordifolia, Gymnema sylvestre, Abieswebna, Vettiveria zizanoides, Ociumum sanctum, Centella asiatica, Trigonellafoenum-graecum, Solanum triblobatum, Azadirachta indica* leaves. This study aims to assess the anticancer properties of Methanolic extract of PHF on breast (MCF7), colorectal (HT29), lung (L2), liver (HepG2), and renal (MDCK) cancer cell lines, as well as its drug-likeness properties through insilico analysis. The methanol extract of PHF was tested for cytotoxic activity using the MTT assay at concentrations ranging from 1000 to 15 µg/ml. The results showed that the compound inhibits the colorectal (HT29) cell line with IC50 values of 97.24 ± 62.04, more effectively than the other four cell lines. Comparatively, the compound isolated from methanolic extract of PHF in previous study demonstrated the best results against colorectal cancer. In the molecular docking investigation, the compound 1,1-Bis(hydroxymethyl)cyclopropane was selected for its interaction with proteins such as Cdk2, TRAF, nitric oxide synthase, Bcl, and

Chk1, which are involved in colon cancer. According to molecular docking and ADMET data, CDK2 exhibited the highest binding energy scores with the compound. These findings suggest that it might be effective against colorectal cancer and further studies using animal model might confirm its anticancerous activity against colorectal cancer.

KEYWORDS: PHF, cell line, Colorectal, molecular docking.

INTRODUCTION

Colorectal cancer, which starts in the colon or rectum, is one of the leading causes of cancer-related deaths worldwide. It typically begins as small, benign polyps that form on the inner lining of the colon or rectum. Over the time, some of these polyps can become cancerous.[1] Bioactive compounds derived from plants can intervene in carcinogenesis at various stages by inhibiting, delaying, or reversing the process before the tumor becomes invasive. Traditional medicines have been used globally to treat cancers due to their anti-angiogenic, anti-invasive, anti-proliferative, antioxidant, anti-inflammatory, and anti-mutagenic properties.[2] The use of natural sources such as berries, grapes, plums, pomegranates, green tea, cruciferous vegetables, soybeans, tomatoes, garlic, turmeric, ginger, olives, whole grains, and mushrooms can inhibit the development of colon carcinogenesis by promoting apoptosis and cell cycle arrest.[3]

However, existing treatment options for colorectal cancer (CRC) are complex and often come with toxic side effects. As a result, researchers are seeking novel drug candidates that exhibit minimal toxicity toward normal, noncancerous cells.[4] To address the issues associated with chemotherapy, radiation therapy, immunotherapy, targeted therapy, and surgery, phytotherapy emerges as an alternative treatment. Phytotherapy utilizes a variety of plant-derived bioactive compounds due to their anti-tumor and chemoprotective activities, as well as their minimal side effects in treating colon cancer.[5] Several secondary plant metabolites, such as flavonoids, phenolics, terpenoids, saponins, quinones, and alkaloids, have demonstrated potent chemoprotective activity against CRC cells by inducing apoptosis and cell cycle arrest.[6]

The combination of plants used in this PHF exhibits good antioxidant activity, antimicrobial activity, antidiabetic activity, anti-inflammatory activity. *Zingiber officinale* suggested as a potential agent for colon cancer.[7] Cuminaldehyde from *Cinnamomum verum* suppressed growth and induced apoptosis, as proved by depletion of the mitochondrial membrane potential, activation of both caspase-3 and -9, and morphological features of apoptosis.[8] Andrographolide, a major constituent of *Andrographis paniculata Nees*, exhibits remarkable

anticancer activity in colon cancer.[9] *Nigella sativa* to exert anti-proliferative, pro-apoptotic, anti-oxidant, cytotoxic, anti-mutagenic, anti-metastatic, and NK cytotoxic activity enhancing effects against various primary cancer cells and cancer cell lines.[10] However, methanolic extract of PHF using *in vitro* cell lines (MCF-7,HepG2,HT29,L2,MDCK)were not evaluated. Hence, the present study aimed to preliminary evaluate the anticancer activity against five cancer cell lines among the best IC50 inhibited cancer protein is carried forward by *in silico* studies for drug likeness and bioavailability.

EXPERIMENTAL

Plant material: Polyherbal formulation (PHF) is a combination of 25 Plant leaves. PHF is procured from Siddha doctor, Madurai, Tamil Nadu.

Extract preparation: The methanolic extract was prepared by soaking 50 g of PHF in 250 mL of methanol using a Soxhlet extractor. The extraction process in a Soxhlet apparatus continues until the solvent becomes colourless.[11] The extracts were concentrated at room temperature, allowing the solvent to evaporate. Then the extract is stored for further use.

Cell culture

MCF-7, HepG2, HT29, L2, MDCK were purchased from TANUVAS(Tamilnadu Vertinary and Animal Sciences University), Chennai, India.

In vitro Anticancer activity

Cytotoxicity effects were determined by the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assay. After 24 hours, a partial monolayer was formed, the medium was removed and cells were treated with different concentrations of compound for 48 hours. Microscopic examination was carried out, and observations were recorded every 12 hours. After the treatment, the solutions in the wells were discarded, washed with PBS (phosphate buffer solution), and 50 µl of freshly prepared MTT (2 mg/ml, prepared in PBS) was added to each well. The plates were shaken gently and incubated for 4 h at 37 °C in a 5% CO_2 atmosphere. After 4 h, the supernatant was removed, and the formazan crystals formed in the cells were solubilized by the addition of 100 µl of DMSO. The plates were shaken gently on a rocker, protected from light, to allow complete solubilization of formazan crystals in DMSO for 30 minutes. Finally, the absorbance was read using a microplate reader at a wavelength of 540 nm. IC50s were calculated for each cancer cell line to determine cytotoxicity.[12,13]

To calculate the percentage of cell viability, the following equation was used: Mean absorbance of treated cells Cell viability (%) 100 Mean absorbance of untreated cells = × For determining the IC50 value, perform a linear regression analysis using the equation: Y = Mx + C, where the value of Y is 50% and the coefficients M and C are derived from the viability graph.

$$\text{Cell viability(\%)} = \frac{\text{Mean absorbance of treated cells}}{\text{Mean absorbance of untreated cells}} \times 100$$

***Insilico* analysis**

Using AutoDock Vina, the proteins from the Colorectal cancer CDK2, TRAF, nitric oxide synthase, Bcl, and Chk1 cancer cell lines were docked with the compound1,1-Bis(hydroxymethyl)cyclopropane of methanolic extract of PHF. The structure of the ligand is retrieved from PubChem database. The ligand structures were used as input for AutoDock Vina docking simulations.[14,15] Crystal structures of the colorectal proteins were retrieved from the Protein Data Bank(PDB) using the IDs 6GUE, 2X7F, 3E7G, 4LXD and 2R0U respectively. The target protein file was generated using AutoDock 4.0's auto preparation feature (MGL tools 1.5.7), which retained the associated protein residue. Protein preparation followed a standard procedure[16], which comprised the removal of co-crystallized ligands, specific water molecules and cofactors. The docking simulation settings were defined using a graphical user interface tool that generated a grid box. The docking algorithm provided by AutoDock Vina was used to determine the optimal docked configuration between ligands and proteins. During the docking process, each ligand had up to nine conformers investigated. PyMOL and Discovery Studio Visualizer were then used to investigate the interactions between ligands and target receptors. The conformations with the lowest free binding energy were chosen for analysis, with interacting residues and hydrogen bonds depicted in stick models and ligands represented in different colours.

ADMET Studies: Analyzing the pharmacokinetic parameters of potential drug candidates is critical in the early phases of drug research. The absorption, distribution, metabolism, excretion and toxicity of chemicals, known as ADMET, were determined utilizing online database Swiss ADME and pk CSM. SwissADME is a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. The selected ligands' SMILES formats were generated from the PubChem database and submitted to the Swiss ADME software of the Swiss Institute of Bioinformatics (http://www. sib.swiss) and the

pkCSM software (http://biosig.unimelb.edu. au/pkcsm/prediction). .The web servers give trustworthy information to analyze physico-chemical parameters such as pharmacokinetics, water solubility, lipophilicity, toxicity and druglikeness.[17] The drug-likeness of compounds was determined using the 'Lipinski's Rule of Five'[18], which indicates the oral bioavailability of selected ligands.

RESULTS AND DISCUSSION

***In vitro* anticancer evaluation:** The MTT method was used to determine the cytotoxicity of PHF. The anticancer activity was evaluated against a variety of cancer cell lines, e.g. MCF-7, HepG2, HT29, L2, MDCK cancer cell lines and the results revealed that the viability (%) decreased as the quantity of isolated molecule increased. The results showed that 1,1-Bis(hydroxymethyl)cyclopropane inhibited many cancer cells in a dose-dependent manner. The percentage cell viability of the compound in cervical cancer varied dramatically depending on the concentration. In HT29 cell line, the compound had a 62.08% viability at 1000µg/ml. However, at 15 µg/mL, the extract exhibited 98.82% cell viability (figure 2) In Hep-G2 cell line, 1,1-Bis(hydroxymethyl)cyclopropane showed a cytotoxic effect at 86.641 µg/ml Table-1.(figure 1)

Table 1: Observed IC50 Value of 1,1-Bis(hydroxymethyl)cyclopropane against cancer cell lines.

Cell Line	IC50 Value(%)
MCF-7	183.91
HT29	86.641
L2	181.01
HepG2	150.43
MDCK	103.71

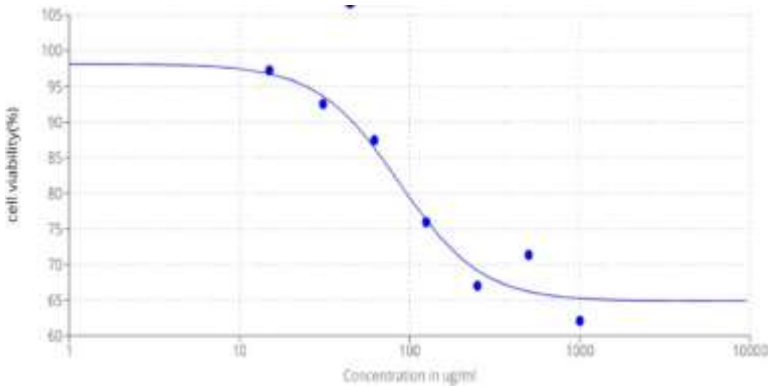

Figure 1: Graphical representation of IC50 (HT29).

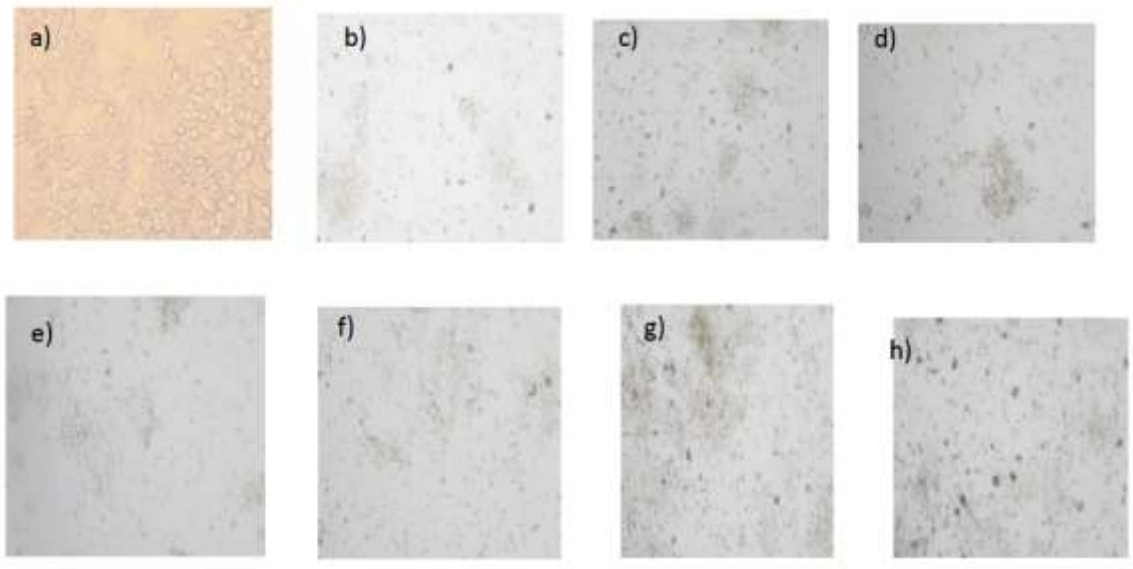

Figure 2: Viability of HT29 cell line treated with 1,1-Bis(hydroxymethyl)cyclopropane in MTT assay (a) control without compound shows 100% viability, (b) 1000µg/mL extract shows 62.08% viability, (c) 500 µg/mL shows 71.33% viability, (d) 250 µg/mL shows 65.96% viability, (e) 125 µg/mL shows 70.53% viability (f) 62 µg/mL shows 72.95% viability g) 31 µg/mL shows 85.97% viability h) 15 µg/mL shows 98.82% viability.

Molecular Docking: The compound previously identified in the active fraction PHF extract was 1,1-Bis(hydroxymethyl)cyclopropane used for this study. As *invitro* anticancer activity showed good cytotoxicity of the compound against colorectal cancer, further docking studies was carried to predict the drug efficiency of this compound. In this study, the compound structure is docked with the proteins namely CDK2, TRAF, nitric oxide synthase, Bcl, and Chk1[19] involved in colorectal cancer. Table 2 presents the docking scores/ interaction of the compound with proteins. The docking score ranges from **-4.3Kcal/mol to -2.8 Kcal/mol.** Thus the compound structure showed potential to interact significantly with CDK2 protein. (Fig. 1) showed that the interactions were stabilized by hydrogen bonds formed by the amino acid residue PRO 352, ASN237 and some alkyl bonds by residue PRO352 and ILE 311.

Table 2: Docking scores with the compound.

Proteins	Binding affinity (Kcal/mol)
CDK2(6GUE)	-4.3
TRAF(2X7F)	-3.9
Nitric oxide synthase(3E7G)	-4.1
Chk1(2R0U)	-2.8
Bcl(4LXD)	-4.0

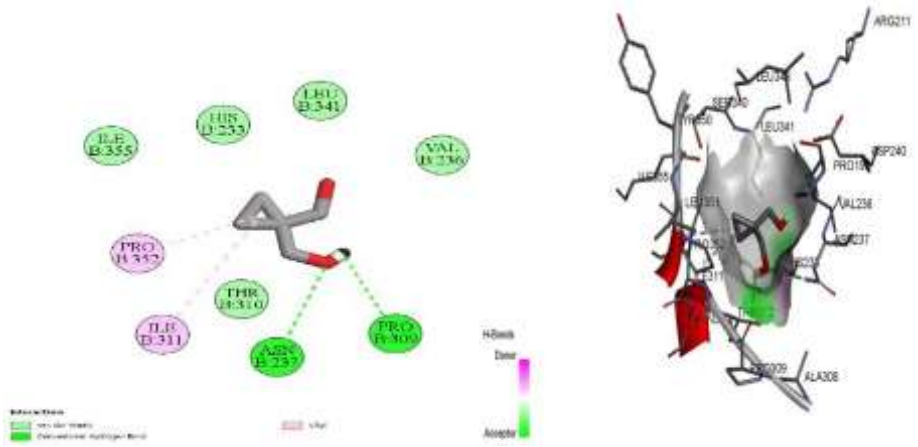

Figure 3: Interactions of 1,1-Bis(hydroxymethyl)cyclopropane within the binding pocket of 6GUE receptor.

Drug-likeness and oral bioavailability analysis: The pharmacokinetic parameters of potential therapeutic candidates should be studied early in the drug discovery process. Lipinski and his team set the following criteria for drug-like compounds: molecular weight (MW) < 500 Da, number of hydrogen bond donors (HBDs) < 5, number of hydrogen bond acceptors (HBAs) < 10 and octanol-water partition coefficient (Log P) < 5. Molecular weight (MW) of the compound 1,1-Bis(hydroxymethyl)cyclopropane is 102.13g/mol. According to Daina & Zoete, the bioavailability radar quickly determines the significant physico-chemical features and drug-likeness of the compound. The pink area in Fig. 4 represents the optimal positioning for each of the bioavailable qualities (LIPO, SIZE, INSOLU, POLAR, INSATU and FLEX) of the compound 1,1-Bis(hydroxymethyl)cyclopropane. The octanol–water partition coefficients (Log P) of the compound is 0.22. Drug candidates exhibit poor absorption when their TPSA(Total polarity surface) is higher than 140 Å2, which is benchmarked for marketed drugs. TPSA value of this compound 1,1-Bis(hydroxymethyl)cyclopropane is 40.46 Å2 and shows high GIA. The water solubility, gastrointestinal absorption (GIA), and blood–brain barrier (BBB) permeability were presented in Table 3. GIA and BBB permeability are important properties of a drug that is intended for widespread use. The potential to inhibit cytochrome P450 (CYP) isoforms was observed for compound 1,1- Bis(hydroxymethyl) cyclopropane (for 5 isoforms); Compound is predicted to show no inhibitory activity against any of the CYP isoforms. From Figure 4 The compound exhibited acceptable GIA and bioavailability. The *insilico* approach allows us to elucidate the binding affinities and binding orientations of the identified compounds, shedding light on their potential as therapeutic agents. Together, these analyses provide valuable insights into the pharmacokinetic and

pharmacodynamic profiles of the investigated compounds, paving the way for drug development.

Figure 4: Bioavailability radar of the 1,1-Bis(hydroxymethyl)cyclopropane.

Table 3: In silico ADMET properties.

Adsorption	**Water Solubility (Log mol/L)**	**Intestinal Human absorption**	**Skin permeation (Log Kp Cm/s)**	**TPSA ($Å^2$)**	
	0(highly soluble)	high	-7.31	40.46	
Distribution	**VDSS(PKcsm)**	**BBB (PKcsm)**	**PPB (PKcsm)**		
	-0.23	0.95	16.51		
Metabolism	**CYP1A2**	**CYP2C19**	**CYP2C9**	**CYP2D6**	**CYP3A4**
	No	No	No	No	No
Excretion	**T ½(half life)**	**Total clearance (Log ML/(Pkcsm) Min/Kg) (Pkcsm)**			
	1.611	1.749			
Toxicity	**HERG inhibitor**	**Acute oral toxicity (Ld50 Mg/Kg) (Pkcsm)**	**Bioavailability**	**Lipinski rule (Swiss ADME)**	
	No	2.001	0.55	No violation	

CONCLUSION

The methanolic extract of PHF yielded a 1,1-Bis(hydroxymethyl) cyclopropane anticancer efficacy against five human cancer cell lines like colon, Lung, liver, renal and breast. The compound had IC50 values of 86.64µg/mL for HT29 (Colorectal), indicating a possible role in the cancer treatment. In molecular studies shows that the compound has good binding affinity with CDK2 protein involved in Colorectal cancer. According to the ADMET parameters, 1, 1-Bis(hydroxymethyl)cyclopropane surpassed its competitors in terms of desirable druglike properties. This compound not only performed well in molecular docking experiments, but it also displayed outstanding biological activity, indicating that it could be a

leading contender for therapeutic development. The convergence of molecular docking data, drug-likeness characteristics and ADMET analysis validates the experimental results. Overall, these results strongly suggest that the identified compound from methanolic extract of PHF hold significant promise as possible therapeutic leads.

ACKNOWLEDGEMENT

The study was technically supported by Dr.G.Thooyavan, from HetroGene Biotech Lab, Chennai 6000008 Tamil Nadu, India. Santhosh ME and Prasanna Kumar S of MSc Bioinformatics from Reva University, Bangalore.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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