Original Article

In silico bioactivity prediction and topical formulation of *Piper longum* root for skin cancer

Saptarshi Samajdar, Debojit Samajdar

From, Department of Pharmaceutical Technology, Brainware University, Kolkata, India.

ABSTRACT

Background: Skin cancer is exceedingly common and the incidence is rising rapidly. Although the mortality rate for skin cancer is not as large as other cancers but still a steady mortality rate around the world remains. As per various reports, a significant number of skin cancer cases have been reported in India mostly from the Northern and Eastern regions. **Objectives:** Although many treatment strategies for skin cancer have been developed over the years, most of them deal with cell toxicity. So, the main objective of this work is to develop a topical formulation using an alternate herbal source ie, *Piper longum* which can provide solution to skin cancer. **Methodology:** A herbal treatment strategy, using *Piper longum* has been demonstrated by an in silico bioactivity study. Further, a set of topical cream formulations using *Piper longum* was prepared and evaluated. **Results:** The study yielded three major compounds, Episesamine (-10.4 kcal/mol), Fargesin (-9.6 kcal/mol) & Pellitorine (-9.1 kcal/mol) from *Piper longum* were highly effective against skin cancer nuclear receptor for Vitamin D (1DB1). The toxicity predicted by the ProTox II tool revealed all ligands with 4-5 level of toxicity and very high LD50 values can be predicted to be safe for human usage. The evaluation of the cream formulation reveals it to be smooth, semisolid nonirritant in nature having high shelf life and spreadability of around 0.3g.cm/s. **Conclusions:** Hence from the results we can conclude that *Piper longum* is predicted to have many phytochemicals with multitude of anti-skin cancer effects and its formulation was also found to be stable, but further, in vitro and in vivo research is required for its usage in humans.

Key words: Skin Cancer, Molecular docking, *Piper longum,* nuclear receptor for Vitamin D, ProTox II, Cream formulation.

The skin provides the outer covering of our body covers over 16% of the body's mass and protects our inside organs from different threats. As the skin is the body's most exposed organ, UV radiation, noxious agents, and toxicants can all harm it. These hazardous factors cause molecular and metabolic stress, resulting in genomic changes in skin cells and skin carcinogenesis [1,2]. Human skin cell transformation into cancer is a multistep process involving initiation, promotion, and advancement that is thought to be stimulated by oxidative stress in cells, resulting in transformation (into cancer), survival, and metastasis. Exposure to ultraviolet radiation (UVR) is a major risk factor for skin cancer. Skin cancers are the most commonly diagnosed malignancies in Western countries, with a significant increase in the Indian subcontinent due to increased exposure to ultraviolet (UV) radiation [3,4]. Skin cancer is defined by an imbalance between inadequate apoptosis and excessive cell proliferation and survival in the epidermis. Although UV radiation is the most common cause of skin cancer, viruses, mutagens in food, mutagens in chemicals, and genetic predisposition are also factors [5].

To control skin cancer, multiple synthetic and mechanical therapeutic strategies have been designed, but many of them reel with the problem of cell toxicity [6]. So, natural products from a highly regarded Ayurvedic plant *Piper longum* can act as a solution to the problem. *P. longum* also called Indian long pepper or pippali, is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning which makes it safe for human use. *Piper longum* is first mentioned in ancient Indian Ayurvedic texts, where its therapeutic and culinary properties are extensively discussed. It

Access this article online

Quick Reponses Code

Received – 14th Apr 2024

Initial Review – 31st May 2024

Accepted – 05th Jun 2024

Correspondence to: Saptarshi Samajdar, Associate Professor, Department of Pharmaceutical, Technology, Brainware University, 398, Ramkrishnapur Road, Kolkata-700125, India. **Email:** saptarshisamajdar1993@gmail.com

arrived in Greece around the sixth or fifth century BCE, although Hippocrates only mentioned it as a spice and not as a medicine [7,8]. In this study, the in-silico evaluation of the skin cancer receptor of *P. longum* is observed as well as preparation & evaluation of an herbal topical formulation has been reported.

MATERIALS AND METHODS

Materials: Authenticated roots of the *P. longum* plant material were acquired from Bixa botanicals and other chemicals were taken from S.D. Finechem & Spectrochem India. Distilled water was used in all the experiments.

Extraction of *P. longum:* *P longum* that had been air-dried and finely powdered was individually added to the maceration pot using petroleum ether, followed by methanol. Under lowered pressure and a regulated temperature, the extract was then concentrated until it was completely dry before being stored in a refrigerator [9].

Ligands preparation and optimization: As reported by Sharma et al., the ligands (9) from *P. longum* root detected using GCMS were reported [10]. Three-dimensional structures of the ligands were created in Open Babel and saved in SDF format for further preparation and molecular docking analysis [11,12].

Preparation and optimization of skin cancer receptor: The protein receptor selected for detection of skin cancer potential was the crystallographic structures of the skin cancer nuclear receptor for Vitamin D (PDB ID: 1DB1), downloaded from the protein data bank. The hetatoms as well as selected water molecules were removed from the protein while adding polar hydrogen for charge using BIOVIA Discovery Studio 2021 visualizer application for its usage in molecular docking [13].

Molecular docking analyses and visualization: The molecular docking studies were performed using PyRx application with Auto dock Vina tool. The protein was firstly saved in .pdb format and loaded to turn it into macromolecule. Then the suitable ligands were also loaded their energy was minimized then using the Auto dock Vina tool the docking study was performed. A grid dimension of 61.06 Å x 51.74 Å x 46.44 Å was chosen for the experiment in order to get the best conforming pattern. The intermolecular interactions between the ligands obtained from *P. longum* and 1DB1 protein were identified and visualized using the Discovery Studio 2021 Client software [14].

Toxicity prediction: The ligands were subjected to toxicity prediction using ProTox II software in human cells (https://tox-new.charite.de/protox_II/). The webserver takes a two-dimensional chemical structure as input and reports the possible toxicity profile of the chemical for 9 models with confidence scores [15].

Formulation of Topical Herbal Cream: White beeswax, liquid paraffin, and *P. longum* were all put into the first beaker. Then, heat on a water bath to ensure even mixing. An oil phase eventually developed after some time. Water, borax, and methyl paraben were added to the second beaker. In a water bath, all the ingredients were boiled together to produce the aqueous phase. The oil phase was continually mixed into the water phase to form a semisolid mass. Once the mixture has become homogeneous, remove it from the heat and let the cream cool. As the liquid-cooled, menthol crystal was added and thoroughly mixed. Two formulations, F1 and F2, were created (**Table 1**) using various beeswax and medication concentrations [16].

Table 1: Formula of herbal cream

Ingredients	F1	F2
P. longum	2.5 g	5g
White beeswax	10 g	7.5g
Liquid Paraffin	22.5 g	22.5g
Borax	0.35 g	0.35g
Water	14.5 g	14.5g
Methyl Paraben	0.15 g	0.15g
Menthol crystal	0.2 g	0.2g

Evaluation of the Cream Formulations: Formulated herbal creams were assessed further using the physical parameters listed below. Physical characteristics color, odor, consistency, and formulation state.Visual inspection allowed for the observation of the color for both formulations. The exact Pantone shade was confirmed by ColorGrab application [17]. The odor of both F1 and F2 formulations was observed by organoleptic evaluations [17]. The state of the cream was visually inspected [17]. The consistency of the formulation was tested by manually rubbing cream on the hand [17]. A digital pH meter (Mettler Toledo 242) was used to measure the herbal cream's pH. 100 ml of distilled water was used to produce the cream solution, which was then left to sit for two hours. pH was determined in three times for the solution and the average value was calculated [18]. The spreadability of the cream formulation was assessed by sandwiching the sample between two slides and compressing it to a constant thickness with a particular weight for a particular period. The specified time required to separate the two slides was measured as Spreadability. The shorter the time taken for separation of two slides results showed better spreadability [18]. To calculate spreadability, use the formula below.

Spreadability = ml/t,
Where,
m-Weight of cream on the slide; l- length moved on the slide; t-time taken

The washability of cream was applied on the skin surface and observed under the running water and the observation was recorded [19]. The non-irritancy test results for an herbal cream formulation were assessed. The preparation lacked irritation and redness [19]. The viscosity of the cream was determined with the help of Brookfield viscometer LDV230 at 6-100 rpm with the spindle no. 63 [19]. The prepared cream was transferred in a suitable wide-mouth container and its phase separation was. Set aside for storage there was not any phase separation occurred between the oil phase and water phase [19]. Accelerated stability testing of prepared formulations was conducted at 40°C ± 1°C for 60 days. The formulations were kept both at room and elevated temperature and observed on the 0th, 10th, 30th, and 60th day for the different parameters [20].

RESULT

In-silico Studies

PyRx docking was utilized to ascertain the binding affinities and significant interactions between *P. longum*-derived phytochemical ligands and the skin cancer nuclear receptor for Vitamin D (1DB1). The binding affinities of the acquired ligands and the standard breast cancer treatment Tamoxifen were evaluated. Table 2 shows the binding affinity obtained from the protein-bound ligands and the standard drug Fluorouracil [21,22]. The binding affinity of the *P. longum* ranged from -9.4 to -4.2 kcal/mol. The molecular interactions between the most active ligands and the active site of the breast cancer receptor-targeting aromatase inhibitor protein were visualized using the Discovery Studio 2021 Client program (**Figure 1**). These samples showed the expected interactions with the protein& active-region amino acids, indicating strong antagonistic characteristics against the aromatase inhibitor protein, which attacks the nuclear receptor for Vitamin D. For the protein coded 1DB1, Episesamine had the highest binding affinity of -10.4 kcal/mol followed by Fargesin (-9.6 kcal/mol) and Pellitorine (-9.4 kcal/mol).

The ligand with values lowest binding affinity was observed in Pipericide (-5.5 kcal/mol). As compared to the standard skin cancer drug Fluorouracil (-7.8 kcal/mol), the binding affinities of three natural compounds (Episesamine, Fargesin, and Pellitorine) derived from *P. longum* were found to be higher indicating their future usage in inhibition of skin cancer (**Table 2**) [23]. The same set of ligands was studied for their toxicity using ProTox II software showed that all the ligands had a predicted class 4 to class 5 toxicity with higher LD50 values indicating their safe usage in humans (**Table 3**) [24]. The development and evaluation of a multi-herbal cream were the focus of the current study. The evaluation criteria for the polyherbal pain relief cream, including its viscosity and phase separation, spreadability, washability, non-irritancy test, and physical examination, were listed under the results in **(Table 4)**.

Table 2: Docking score of *P.longum* ligands

Ligands	Binding Affinity (ΔG in kcal/mol)
	1DB1
Brachystamide	-7.4
Caryophyllene	-7.2
Episesamine	-10.4
Fargesin	-9.6
Pellitorine	-9.1
Pipericide	-5.5
Piperitine	-5.7
Piperine	-5.9
Piperlongumine	-6.7
Flurouracil	-7.8

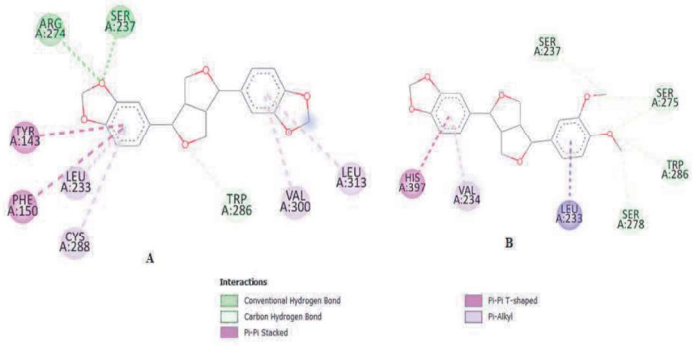

Figure 1: Interaction diagram of A. Episesamine B. Fargesin

Table 3: Toxicity prediction of ligands

Ligands	Level of Toxicity (1=highly toxic; 6= safe)	Predicted LD50 (µg/ml)
Brachystamide	4	760
Caryophyllene	5	5300
Episesamine	4	1500
Fargesin	4	1500
Pellitorine	5	4185
Pipericide	4	760
Piperitine	4	380
Piperine	5	3100
Piperlongumine	4	1160

Evaluation of topical formulation

The color of F1 formulation was observed to be brown orange (84831F) and greenish orange (812417F) for F2. The odor of the ayurvedically important herbal topical formulations was observed to have having common characteristic cream and paste smell. Thus this ensures the stability of the formulations with no

rancid smells. Both the F1 and F2 formulations were shown to be in a semisolid state. Their appearance was mostly showing plastic flow properties [25-27]. The consistency of the herbal topical formulations was seen to be smooth [25]. The pH of the F1 cream formulation was found to be 7.2 while the pH of F2 formulation was found to be 7.06, both of which are neutral with F2 having a better pH. No change of pH was observed even after four months of testing [28]. It was found that the cream was easily spreadable and had moisturizing properties. The exact values of spreadability were found to be 0.3±0.06 g.cm/s and 0.26±0.04 g.cm/s for F1 and F2 respectively. The spreadability value remains the same even after 4 months of observation [28].

Table 4: Results of polyherbal cream

Parameter	F1	F2
Color	Brown orange (84674F)	Pale Brown orange (84831F)
Odor	Characteristic smell	Characteristic smell
State	Semisolid	Semisolid
Consistency	Smooth	Smooth
pH	7.20	7.06
Spreadability	0.3±0.06 g.cm/s	0.26±0.04 g.cm/s
Washability	Sticky	Non-Sticky
Non-irritancy test	Non-irritant	Non-irritant
Viscosity	2803±0.32cps.	2689±0.18cps
Phase separation	No phase separation	No phase separation

The washability of F1 formulation was shown to be sticky but F2 got easily removed from the skin surface thereby showing F2 had higher washability, indicating no stain or sticky mass on the skin [29]. In irritancy studies, it was discovered that none of the formulations cause redness, edema, inflammation, or irritation of the skin. There was no sign of redness or other ailments even after 4th month of storage. The viscosity of formulated cream was determined by Brookfield viscometer at 20 rpm using spindle no. 63. The viscosity of cream has been reported in the range of 2000 to 4999 cps which indicates that the cream is easily spreadable by a small amount of shear. The formulated creams F1 and F2 show a viscosity of 2803±0.32cps and 2689±0.18cps respectively [30]. The creams were very stable without any kind of phase separation observed. Moreover, no phase separation was seen even after four months of storage. There were no observable changes in any of the parameters for the topical cream formulations (F1 and F2) even after 60 days of stability testing [19].

DISCUSSION

Skin cancer is very frequent and is becoming more commonplace. Even while skin cancer mortality is not as high as that of other cancers, skin cancer mortality is nonetheless consistently high worldwide. Several sources state that a considerable number of skin cancer cases, primarily from the Northern and Eastern parts of India, have been documented. So, to combat this, a robust treatment strategy is the need of the hour. Although there are various synthetic strategies available but most of them faces issue of toxicity and multi drug resistance. So, a new strategy using *P. longum* root can find better potential. The phytochemicals from macerated extracts were selected for molecular docking studies.

From the set of phytochemicals, three phytochemicals Fargesin (-9.6 kcal/mol), Episesamine (-10.4 kcal/mol), and Pellitorine (-9.1 kcal/mol) showed maximum skin cancer activity even higher than the standard Flurouracil. The toxicity prediction data showed the extracts to be safe for human use. Thus, from these data it can be a promising anticancer treatment strategy [31]. Based on these results, a set of topical formulation was designed and the evaluation of topical formulation revealed that the color to be of brown color, having semisolid state. The pH of the formulation F1 was 7.2 while the pH of F2 was 7.06 indicating F2 having a better pH. With F2 having a lower viscosity and no phase separation and nonirritant, non-sticky was selected as a better formulation [32]. Thus with further studies like in vitro or in vivo as well as clinical trial, the treatment strategies can be established [33].

CONCLUSION

Hence from the *in-silico* studies it could be observed that multiple compounds of *P. longum* root like Fargesin, Episesamine, and Pellitorine found in *P. longum* root showed better binding affinity as compared to the standard drug in skin cancer receptors. All the ligands were found to be safe (Grade 4-5) in toxicity prediction studies. Overall the compounds showed promising results for their usage in skin cancer nuclear receptors for Vitamin D. So, using the promising extract topical cream-based formulations were prepared which were in the range of neutral pH and had no irritancy. The viscosity (2689±0.18cps-2803±0.32cps) and spreadability values had promises for its wide use on skin. Moreover, no changes were observed in 60-day accelerated stability studies, indicating their superior shelf life. Further in vitro and in vivo studies are required to provide evidence of its usage in humans.

REFERENCES

1. D'Orazio J, Jarrett S, Amaro-Ortiz A, et al. UV radiation and the skin. Int J Mol Sci. 2013; 14(6):12222-48.
2. Pustišek N, Šitum M. UV-radiation, apoptosis, and skin. Coll. Antropol. 2011; 35(2):339-41.

3. Labani S, Asthana S, Rathore K, et al. Incidence of melanoma and nonmelanoma skin cancers in Indian and the global regions. J Cancer Ther. 2021; 17(4):906-11.
4. Leiter U, Keim U, Garbe C. Epidemiology of skin cancer: update 2019. Sunlight, vitamin D, skin cancer. 2020:123-39. Available from: https://doi.org/10.1007/978-3-030-46227-7_6.
5. Gandhi SA, Kampp J. Skin cancer epidemiology, detection, management. Med Clin. 2015; 99(6):1323-35.
6. Payne AS, James WD, Weiss RB. Dermatologic toxicity of chemotherapeutic agents. InSeminars in oncology 2006; 33(1):86-97.
7. Yadav V, Krishnan A, Vohora D. A systematic review on *Piper longum* L. Bridging traditional knowledge and pharmacological evidence for future translational research. J Ethnopharmacol. 2020;247:112255.
8. Wasti A, Pant KK. Callus Induction from the Leaves and Organogenesis from the Leaf induced Calli of *Piper longum* L. Int. J Biores Stress Manag. 2019; 10(3):287-91.
9. Bhawar SB, Dighe SB, Tambe RB. Formulation and evaluation of polyherbal ointment for wound healing and antimicrobial activity. J Drug Deliv Ther. 2019; 9(3-s):575-82.
10. Sharma V, Kalyani R, Vyas P, et al. Pharmacognostical and phytochemical study of *Piper longum* L. and *Piper retrofractum* Vahl. J Pharmaceu Scien Innova. 2012; 1(1): 62-66.
11. Srivastava V, Yadav A, Sarkar P. Molecular docking and ADMET study of bioactive compounds of *Glycyrrhiza glabra* against main protease of SARS-CoV2. Mater. Today Proc. 2022; 49:2999-3007.
12. Rehman A, Ashfaq UA, Javed MR, et al. The Screening of phytochemicals against NS5 Polymerase to treat Zika Virus infection: Integrated computational based approach. Combinatorial Chemistry & High Throughput Screening. 2022; 25(4):738-51.
13. Rut W, Lv Z, Zmudzinski M, et al. Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti–COVID-19 drug design. Sci Adv. 2020; 6(42):eabd4596.
14. Adedotun IO, Abdul-Hammed M, Hamzat BA, et al. Molecular docking, ADMET analysis, and bioactivity studies of phytochemicals from *Phyllanthus niruri* as potential inhibitors of hepatitis C virus NSB5 polymerase. J. Indian Chem. Soc. 2022; 99(2):100321.
15. Navindgikar NN, Kamalapurkar KA, Chavan PS. Formulation and evaluation of multipurpose herbal cream. Int. J Curr Pharm Res. 2020; 12(3):25-30.
16. Ubani A, Agwom F, Shehu NY, et al. Molecular docking analysis of some phytochemicals on two SARS-CoV-2 targets. BioRxiv. 2020. https://doi.org/10.7910/DVN/BB0QQK.
17. Mishra AP, Saklani S, Milella L, et al. Formulation and evaluation of herbal antioxidant face cream of Nardostachys jatamansi collected from Indian Himalayan region. Asian Pac J Trop Biomed. 2014; 4:S679-82.
18. Vigneshwaran LV, Swetha KA, Sivakumar S, et al. Formulation and evaluation of herbal face cream with Green tea extract. Int J Res Pharm Sci Tech. 2022; 3(1):1-4.
19. Matangi SP, Mamidi SA, Raghavamma ST, et al. Formulation and evaluation of anti aging poly herbal cream skin. Int J Pharm Sci Rev Res. 2014; 5(6):133-136.
20. Dhyani A, Chander V, Singh N. Formulation and evaluation of multipurpose herbal cream. J Drug Deliv Ther. 2019; 9(2):341-3.
21. Samajdar S, Mondal P. In silico studies on the phytochemical components of *Lagenaria siceraria* targeting aromatase receptors against breast cancer. In Silico Pharmacol. 2023; 11(1):19.
22. De A, Bhattacharya S, Debroy B, et al. Exploring the pharmacological aspects of natural phytochemicals against SARS-CoV-2 Nsp14 through an in silico approach. In Silico Pharmacol. 2023; 11(1):12.
23. Lakhera S, Devlal K, Ghosh A, et al. In silico investigation of phytoconstituents of medicinal herb '*Piper Longum*'against SARS-CoV-2 by molecular docking and molecular dynamics analysis. Results Chem. 2021; 3:100199.
24. Dhorajiwala TM, Halder ST, Samant LR. Computer-aided docking studies of phytochemicals from plants Salix subserrata and Onion as inhibitors of glycoprotein G of rabies virus. Biomed Biotech Res J. 2019; 3(4):269-76.
25. Valarmathi S, Kumar MS, Sharma V, et al. Formulation and Evaluation of Herbal Face Cream. Res J Pharm Tech. 2020; 13(1):216-8.
26. Iriventi P, Gupta NV. Formulation and Evaluation of herbal cream for treating psoriasis. Res J Pharm Tech. 2021; 14(1):167-70.
27. Dhase AS, Khadbadi SS, Saboo SS. Formulation and evaluation of vanishing herbal cream of crude drugs. Am J Ethnomed. 2014; 1:313-8.
28. Sonalkar MY, Nitave SA. Formulation and evaluation of polyherbal cosmetic cream. World J Pharm Pharm Sci. 2016; 5:772-9.
29. Sirsat SV, Rathi NM, Hiwale AS, et al. A review on preparation and evaluation of herbal cold cream. World J Pharm Res. 2022; 11(5):690-7.
30. Satpute KL, Kalyankar TM. Development and evaluation of herbal cream for the treatment of acne. J Pharmacogn Phytochem. 2019; 8(3):2618-24.
31. Wawszczyk J, Wolan R, Smolik S, et al. In vitro and in silico study on the effect of carvedilol and sorafenib alone and in combination on the growth and inflammatory response of melanoma cells. Saudi Pharm J 2023; 31(7):1306-16.
32. Okafo SE, Anie CO, Alalor CA, et al. Evaluation of physicochemical and antimicrobial properties of creams formulated using Pterocarpus santalinoides seeds methanol extract J Appl Pharm Sci. 2023; 13(5):126-35.
33. Mlozi SH, Mmongoyo JA, Chacha MN. In vitro evaluation of the herbal cream formulation from methanolic leaf extracts of Tephrosia vogelii Hook. f for topical application. Clin Phytosci. 2023; 9(1):3.

How to cite this article: Saptarshi Samajdar, Debojit Samajdar. *In silico* bioactivity prediction and topical formulation of *Piper longum* root for skin cancer. Indian J Pharm Drug Studies. 2024; Online First.

Funding: None; *Conflicts of Interest: None Stated*