



## **Anti-Cancer Effect of Novel Hydroxy Flavons on Human Cancer Cell *In vitro* and *In silico***

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### **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

Anti-cancer effect of 7 hydroxy Flavone and its derivatives were tested against human cancer cell lines such MCF-7 cell line using the *in vitro* by MTT assay in cancer cell lines. All the flavones showed significant activity against all pathogens. 7-HF showed a maximum zone of inhibition against cancer cell lines. 7-try hydroxy flavones showed the lowest anti-cancer activity. And docking results shown as 7-HF has shown maximum docking -7.9, 7,8,3'-THF has -7.5 and 7,3',4' has -7.7. as per the results, 7 hydroxy flavone could be a possible source to obtain new and effective compounds to treat cancer.

**Keywords:** MTT assay; MCF-7; hydroxy flavones; Tamoxifen; *In silico*.

### **1. INTRODUCTION**

The growth or identification of compounds gifted in killing transformed or cancer cells, without being toxic to their usual counterparts, is of utmost importance and has the increasing interest of scientists universal. Since ancient times, plants have been considered rich sources of chemicals, with immense therapeutic potential.

During current years, some of these natural plant-derived compounds or phytochemicals have been exposed to be highly capable anticancer mediators, in addition to being real against many other diseases [1]. Cancer, following cardiovascular diseases, is the main cause of mortality & morbidity in Europe. Specifically, only in Europe, approximately 3.45 million new cases of cancer were reported in

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2012, excluding non-melanoma skin cancer, whereas roughly 1.75 million deaths happened [2] Flavonoids and phenolics, in specific, represent an important element of a normal human food [3]. The usual day-to-day flavonoid intake varies from nearly 1-2 g per day [4]. The polyphenolic compounds have been reported to have many pharmacological activities, such as antioxidant, anti-inflammatory, anticarcinogenic, antiviral, or antiallergic effects. Among anticancer and cancer-preventing drugs, flavonoids are the most studied ones. These compounds can interfere with specific stages of the carcinogenic process, inhibit cell proliferation and induce apoptosis in several types of cancer cells [5].

Molecular docking (or simply docking) is an in silico method used to analyze the interactions between two molecules. Of the two molecules, one will act as a test compound or ligand, while the other will act as a target or known as a receptor. In its use, the docking method is widely used in various purposes in the field of drug design and discovery, especially for screening in the discovery of potential compounds with certain potential activities, as well as to explain the mechanism of action of the interactions that occur between drug compounds with known activity against the target protein [6]. Compared to several other in silico methods, molecular docking is one of the most popular and widely used, both as the primary method and for confirming other methods. From the beginning of 2020 to October 2020 alone, molecular docking presents various challenges in its analysis. These obstacles are generally related to the type of software used, considering that much software can be used to perform molecular docking, both free and paid. Apart from the technical problems associated with the software used, one of the biggest challenges in analyzing the docking results is the ligand ranking based on the docking result [7].

Based on this background, this study aims to introduce a new way to analyze docking results with a two-dimensional graph between the difference in docking score and the similarity of ligandreceptor interactions.

## 2. MATERIALS AND METHODS

### 2.1 Collection and Identification of Flavonoids

This 7hydroxy flavone and its derivatives were selected for the study and these were obtained

from Sigma Aldrich, USA. The test compounds were prepared as a fine in power.

#### 2.1.1 Drugs and chemicals

Streptomycin (Hindustan Antibiotics Ltd), Amphotericin B (Abbott), Penicillin (Abbott), Tamoxifen (Zydus Pharma).

### 2.2 MTT Cell Proliferation Assay

Cell lines and Culture medium: MCF-7 (Human breast adenocarcinoma) were procured from National Centre for Cell Sciences (NCCS), Pune, India. It is a suspension culture and stock cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL) and amphotericin B (5 µg/mL) in a humidified atmosphere of 5% CO<sub>2</sub> at 37 °C until confluent. The confluent cell suspension was centrifuged at 2000 rpm for 10 min and the cell pellet was resuspended in a fresh medium. The stock cultures were grown in 25 cm<sup>2</sup> culture flasks and all experiments were carried out in 96 microtiter plates.

### 2.3 MTT Assay for Cell Viability

The MTT assay is based on the ability of live but not dead cells to reduce a yellow tetrazolium dye to a purple formazan product. Cells were maintained in DMEM medium, supplemented with 10% Fetal Bovine Serum, at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10<sup>4</sup> cells/well and allowed to attach overnight at 37°C. The medium was then discarded and cells were incubated with different concentrations of the extracts (10,20,40,60,80,100,120,140,) for 24 hours.

After the incubation, the medium was discarded and 100µl fresh medium was added with 10µl of MTT (5mg/ml). After 4 hours, the medium was discarded and 100µl of DMSO was added to dissolve the formazan crystals. Then, the absorbance was read at 570nm in a microtitre plate reader. Tamoxifen was used as a positive control.

### 2.4 Molecular Docking

#### 2.4.1 Materials ligands preparation

As a representative of the test ligands the test ligand structure was obtained from the PubChem

(<https://pubchem.ncbi.nlm.nih.gov/>) and then downloaded in SDF format. All test ligands were then prepared according to the method reported by then saved in .pdbqt format and was downloaded from the Protein Data Bank website (<https://www.rcsb.org/>). The receptor consists of three chains (A, B, and C), with the chains used for the docking process was chain A. The parts of the receptors that were not used (e.g., water, solvent, unused chains) were then removed and given polar hydrogen as well as charges— and finally saved in .pdbqt format using Auto Dock Tols.

## 2.5 Methods

The hardware and software used in this study were the same as the research reported by Pratama et al.[8], with Auto Dock Vina for docking and Discovery Studio Visualizer for visualization. Twodimensional graphical creation of ligandreceptor interactions was carried out using Microsoft Excel 2019.

The docking protocol validation was carried out by the redocking method [8]. The observed parameter was a rootmeansquare deviation (RMSD). The RMSD value less than 2 Å indicating a valid docking protocol and can be used for the docking process.

Molecular docking for all test ligands performed in the same way as the validation process with similar sizes and positions of the grid box. The results were grouped under two parameters: free energy of binding ( $\Delta G$ ; kcal/mol) and ligand receptor interactions. The ligandreceptor interactions are recorded based on two parameters: the amino acids that interact and the types of interactions that occur. The docking process was repeated five times, and the average  $\Delta G$  value and the deviation were determined. The maximum allowable deviation value was  $\pm 0.05$  kcal/mol to avoid high variation. calculated and expressed as a percentage.

Twodimensional graph of ligandreceptor interactions the difference in  $\Delta G$  values and the ligandreceptor interactions obtained earlier was then used to create a twodimensional graph. The x axis was filled with the reduction in the  $\Delta G$  value of each test ligand against the  $\Delta G$  value of the reference ligand (celecoxib). The difference from the  $\Delta G$  value of each test ligand against  $\Delta G$  flavone was calculated based on the following Equation:

$$\Delta G_{dif} = \Delta G_{test} - \Delta G_{ref}$$

$\Delta G_{dif}$  = the difference from the  $\Delta G$  of test and reference ligand

$\Delta G_{test}$  =  $\Delta G$  of test ligand

$\Delta G_{ref}$  =  $\Delta G$  of reference ligand

## 3. RESULTS

MTT assay indicated that flavonoids with different chemical structures showed a differential cytotoxic effect on cancer cells was recorded (Fig 1). 7-HF, 7,3',4'-THF and 7,4',8'-THF these hydroxyflavanones was exhibited the most potent cytotoxic effect on cells. Nearly 77% inhibited with **7-HF**, and **7,3',4'-THF** recorded strong cytotoxicity comparatively 7,8,3'THF in cells, in high concentration **7-HF recorded 77%**, and **7,3',4'-THF** recorded 75% strong cytotoxicity, and 7,8,3'THF was recorded 69 %. It was recorded in low concentration 10 $\mu$ g/ml, 7-HF 12.38%, 7,3',4'-THF 17.29% and 7,8,3'-THF ahs 8.86% cell inhibition.

The IC<sub>50</sub> values recorded for the above hydroxy flavones are as follows

$$\begin{aligned} 7, 8, 3' - \text{THF} &= 106.58 \mu\text{M} \\ 7, 3', 4' - \text{THF} &= 79.15 \mu\text{M} \\ 7 - \text{HF} &= 86.88 \mu\text{M} \end{aligned}$$

## 4. DISCUSSION

Flavonoids belong to a chemically heterogeneous group of small molecules with chemopreventive activity. They exert specific cytotoxic activity towards cancer cells which has generated large interest in developing flavonoid based cytostatics for anti-cancer therapy. Previous studies have demonstrated significant anti-cancer activity in some natural flavonoids such as apigenin [9], genistein, quercetin [10] and luteolin [11]. The present study indicated that 7-hydroxy flavone exhibited the most potent cytotoxic effects on these cancer cells among the flavonoids tested, suggesting that 7-HF may have stronger anti-cancer activity than any of the above-mentioned flavones. It was an exciting finding because the anti-cancer activity of 7-HF was never reported previously. To develop compounds with anti-cancer and other pharmacological properties from natural substances has become a focus of interest<sup>45</sup>. 7-hydroxy flavone may serve as a leading compound for developing more potent anti-cancer drugs. In addition, it is noteworthy that 7-HF showed unique anti-cancer properties. All of the 3 flavanones tested in this study, except 7,3',4'-THF flavanone, showed slight less

cytotoxic effects. The peculiar anti-cancer activity of 7-HF flavanone needs further study. The results also indicated that flavonoids such as 7,3',4'-THF and 7,8,3' THF showed also moderate significant cytotoxic effects. The findings suggest that not all-natural flavonoids possess beneficial cancer chemopreventive properties and that some of them may even have adverse effects on the prevention and treatment of cancer. Different mechanisms have been linked to flavonoid mediated cytotoxicity, including proteasome inhibition [12], inhibition of fatty acid synthesis, topoisomerase inhibition

[13], induction of cell cycle arres, accumulation of p53 [14] or enhanced expression of c-fos and c-myc [15]. As multiple mechanisms account for flavonoid-induced cytotoxicity, the development of structure-activity relationships to predict the cytotoxic potential of a given compound may facilitate the search for effective candidates for cancer therapy. In this study, we identified 7-HF as an active anti-cancer compound that works through apoptosis induction. These findings may be useful for developing potent anti-cancer compounds from flavonoids for potential clinical applications.

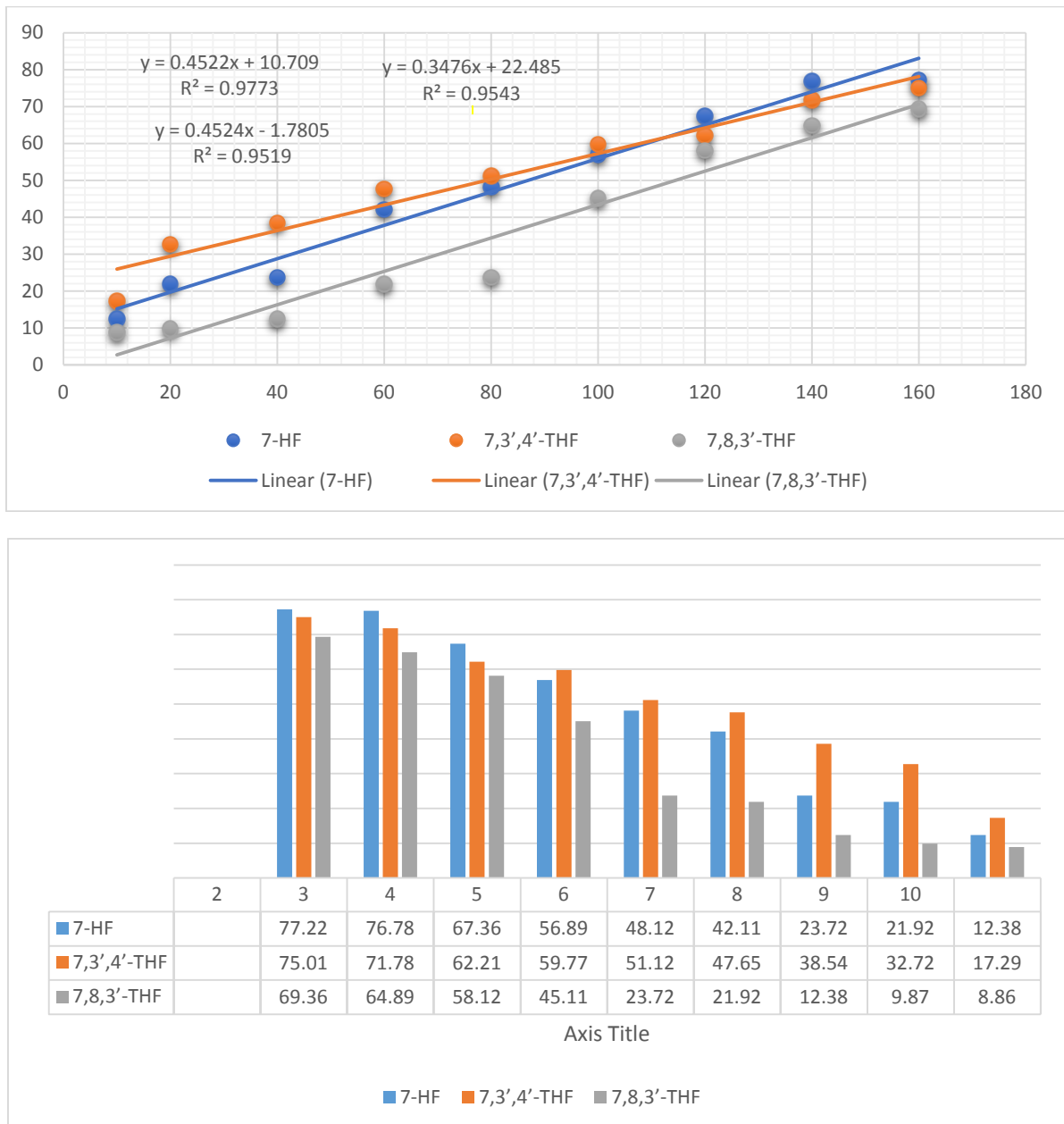


Fig. 1. *In vitro* MTT assay cytotoxicity effect of test substance 7-HF, 7,3',4'-THF and 7,8,3'-T

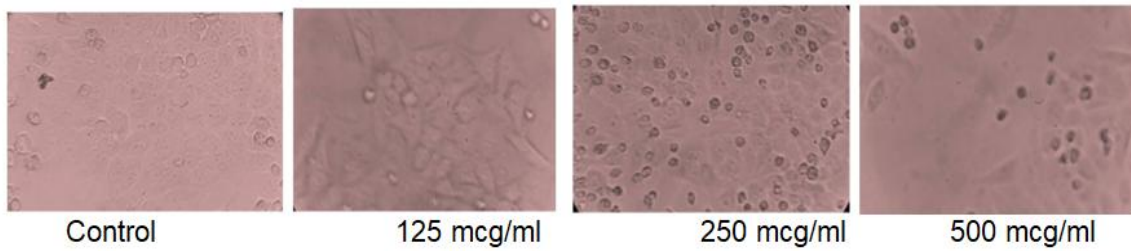


Fig 2. *In vitro* (MTT assay) cytotoxicity effect of test substance 7-HF against MCF-7 cell line

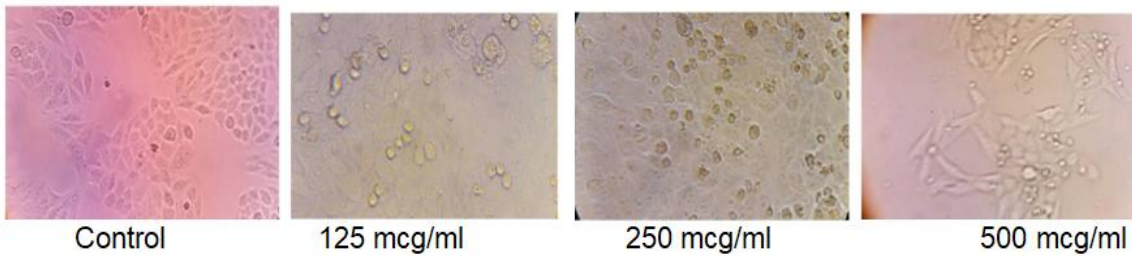


Fig 3. *In vitro* (MTT assay) cytotoxicity effect of test substance 7,3',4'THF against MCF-7 cell line

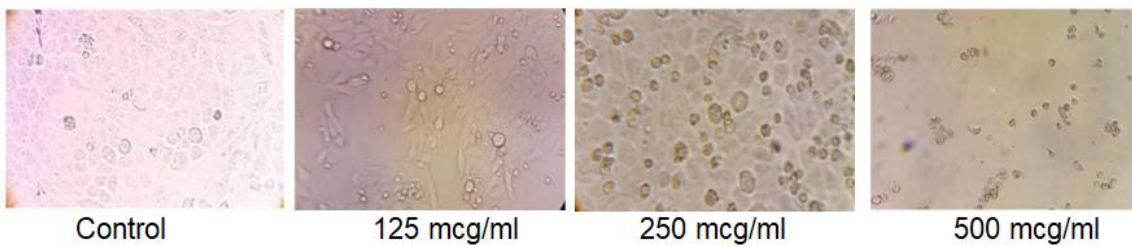


Fig 4. *In vitro* (MTT assay) cytotoxicity effect of test substance 7,8,3'THF against MCF-7 cell line

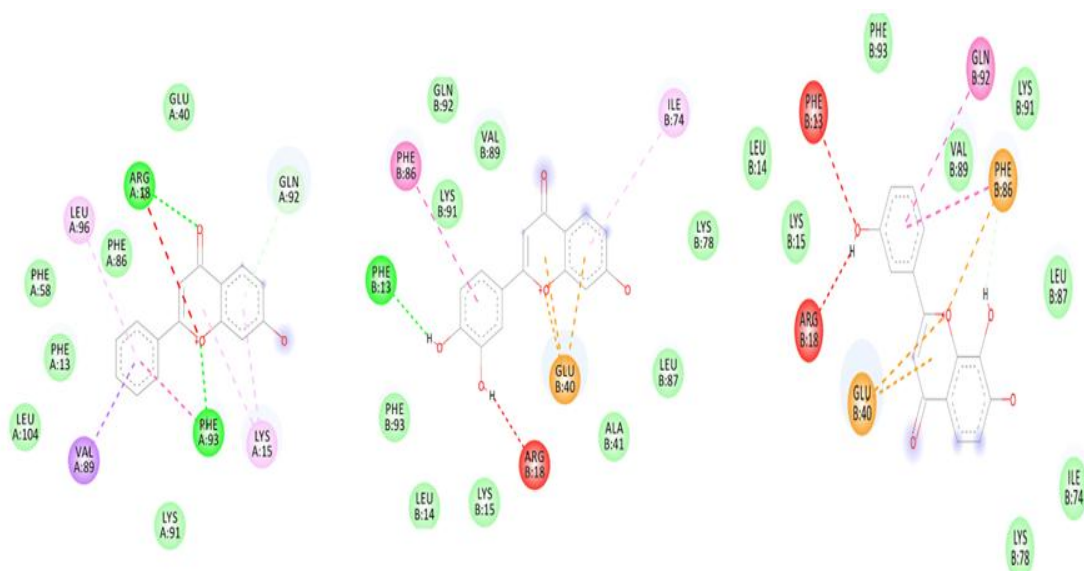


Fig. 5. Interactions of 7-HF, 7,8,3'-THF, and 7,3',4' THF with Survivin (1E31)

**Table 1. Docking score of 7-hydroxyflavone, 7,3',4'-trihydroxyflavone, 7,8,3'-trihydroxyflavone with 1E31**

Flavones	Docking score with 1E31	h-bonds
7-HF	-7.9	ARG'18 PHE'93
7,3',4'-THF	-7.7	PHE'13 ARG'18
7,8,3'-THF	-7.5	PHE'13 ARG'18

#### 4.1 Molecular Interactions OF 7-HF, 7,3',4'-THF, 7,8,3'-THF WITH 1E31

7-HF, 7,3',4'-THF, 7,8,3'-THF were docked with Survivin (1E31).

The ligand 7-HF showed interactions with LYS'15, ARG'18, VAL'89, GLN'92, PHE'93, and LEU'96. Conventional hydrogen bonding was seen with ARG'18 and PHE'93 amino acid residues of the protein Survivin (1E31).

The ligand 7,3',4'-THF showed interactions with PHE'13, ARG'18, GLU'40, ILE'74 and PHE'86. Conventional hydrogen bonding was seen with PHE'13 and ARG'18 amino acid residues. Pi interactions with GLU'40, ILE'74 and PHE'86 amino acid residues of the protein Survivin (1E31).

The ligand 7,8,3'-THF showed interactions with PHE'13, ARG'18, GLU'40, PHE'86, and GLN'92 amino acid residues of protein Survivin (1E31). Conventional hydrogen bonding was seen with PHE'13 and ARG'18. Pi interactions was seen with GLU'40, PHE'86, and GLN'92 amino acid residues of protein Survivin (1E31).

#### 5. CONCLUSION

In conclusion, the present study confirms the MTT assay and docking studies of flavone derivatives. These findings will open a new channel to synthesize halogenated flavones and explore the development of synthetic flavones derivatives for the treatment of a wide range of diseases associated with cancer.

#### DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not

intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

#### CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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