Original Research Article

Anti-cancer effect of novel Hydroxy Flavons on Human Cancer Cell *in Vitro and in silico*

Abstract: The present study focused on the elucidation of the putative anticancer potential of 7HF,7,3',4'THF and 7,4',8THF. The anticancer activity of These novel hydroxy flavones at **10, 20, 40,60, 80,120** and 140µM was assessed in vitro by MMT assay in cancer cell lines.in this 4'THF and 7,4',8THF shown cell viabitly but 7-HF having good Cell Viability when comparing with 4'THF and 7,4',8THF as shown in result table. and IC_{50} **values are** 7-HF has 86.88, 7,3',4'-THF has 79.157 and 7,8,3'-THF has 106.58. in silico also having 7-HF(7.9) 7,3',4'-THF(-7.7) and 7,8,3'-THF(-7.5) good docking score.

Keywords: MMT assay, MCF-7, Hydroxy flavone, Tamoxifen. In silico.

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 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.

MATERIAL AND METHODS

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.

Drugs and chemicals: Streptomycin (Hindustan Antibiotics Ltd), Amphotericin B(Abbott), Penicillin(Abbott), Tamoxifen (Zydus Pharma).

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% CO2 at 37 ºC 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 cm2 culture flasks and all experiments were carried out in 96 microtiter plates.

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^{o}C in a humidified atmosphere with 5% CO_2. The cells were plated in 96 well flat bottom tissue culture plates at a density of approximately 1.2 X 10^4 cells/well and allowed to attach overnight at 37^{o}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.

MOLECULAR DOCKING

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 four 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 AutoDockTools

Methods The hardware and software used in this study were the same as the research reported by Pratama et al. (2020), with AutoDock 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 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 (ΔG; kcal/mol) and ligand receptor interactions. The ligand-receptor 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 ΔG value and the deviation were determined. The maximum allowable deviation value was ±0.05 kcal/mol to avoid high variation.calculated and expressed as a percentage.

Twodimensional graph of ligandreceptor interactions The difference in ΔG 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 ΔG value of each test ligand against the ΔG value of the reference ligand (celecoxib). The difference from the ΔG value of each test ligand against ΔG flavone was calculated based on the following Equation

$$\Delta Gdif = \Delta Gtest - \Delta Gref$$

ΔGdif = the difference from the ΔG of test and reference ligand
ΔGtest = ΔG of test ligand
ΔGref = ΔG of reference ligand

RESULTS:-

MTT assay indicated that flavonoids with different chemical structures showed a differential cytotoxic effect on cancer cells was recorded (Fig I). 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 10µg/ml, 7-HF 12.38%, 7,3',4'-THF17.29% and 7,8,3'-THF ahs 8.86% cell inhibition.

The IC_{50} values recorded for the above hydroxy flavones are as follows

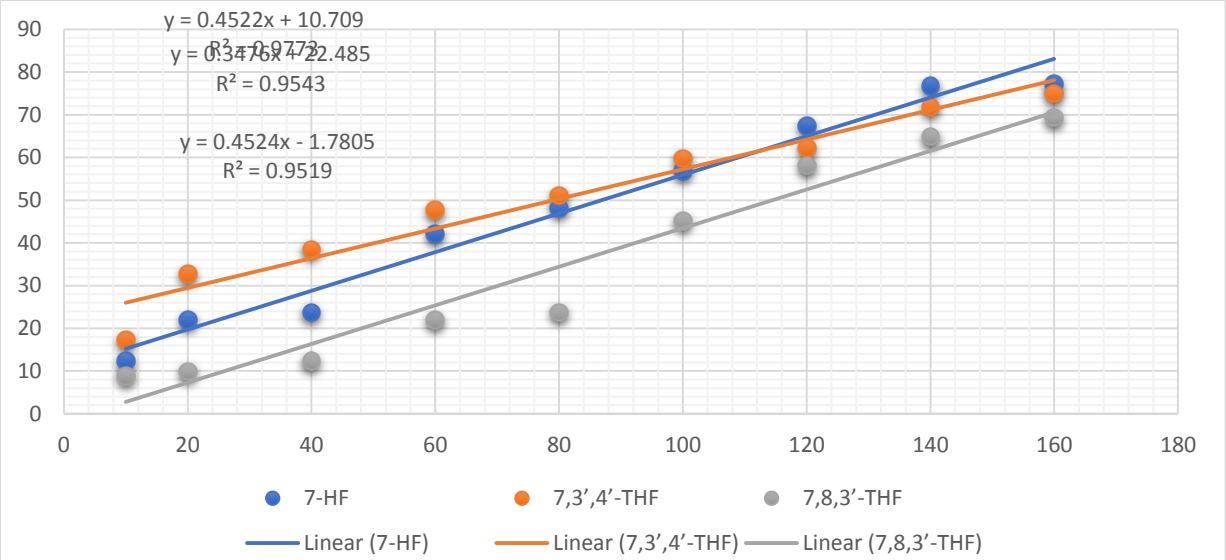
7, 8, 3' – THF = 106.58 µM

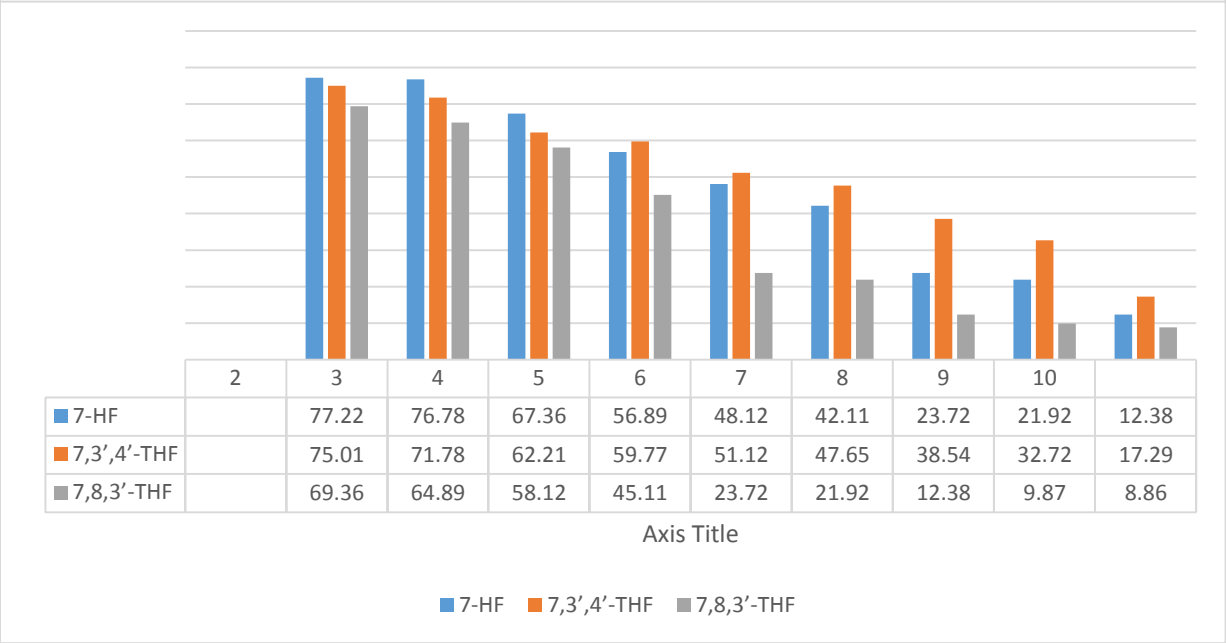
7, 3', 4' – THF = 79.15 µM

7 - HF = 86.88 µM

Fig- I

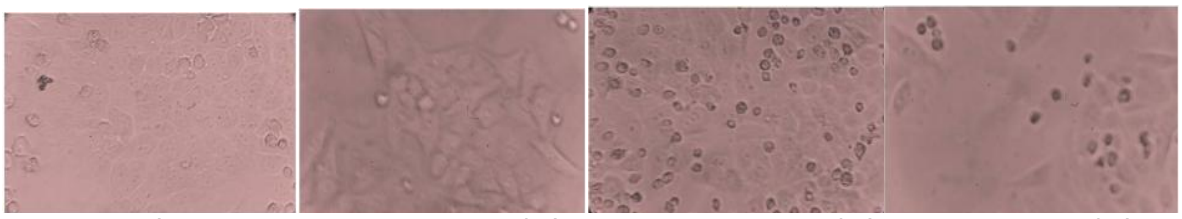
In vitro MTT assay cytotoxicity effect of test substance 7-HF, 7,3',4'-THF and 7,8,3'-T





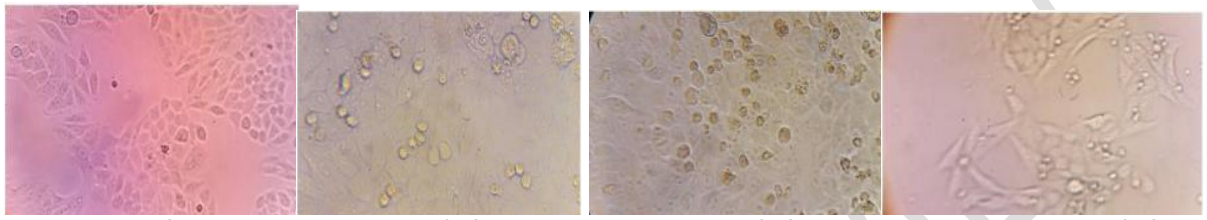
	2	3	4	5	6	7	8	9	10	
7-HF		77.22	76.78	67.36	56.89	48.12	42.11	23.72	21.92	12.38
7,3’,4’-THF		75.01	71.78	62.21	59.77	51.12	47.65	38.54	32.72	17.29
7,8,3’-THF		69.36	64.89	58.12	45.11	23.72	21.92	12.38	9.87	8.86

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



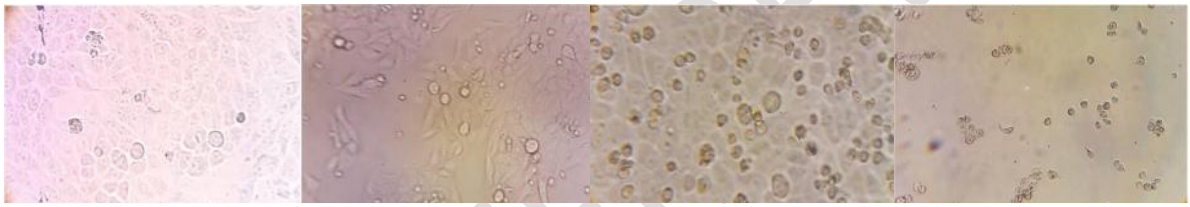
Control 125 mcg/ml 250 mcg/ml 500 mcg/ml

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



Control 125 mcg/ml 250 mcg/ml 500 mcg/ml

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



Control 125 mcg/ml 250 mcg/ml 500 mcg/ml

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

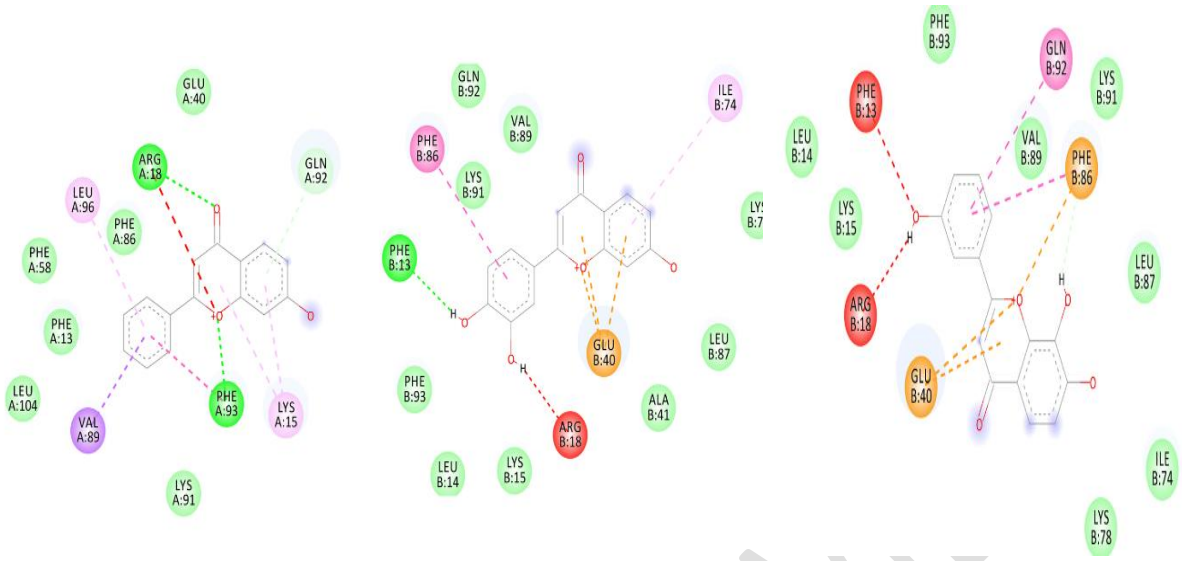


FIGURE 5: Interactions of 7-HF, 7,8,3'-THF, and 7,3',4' THF with Survivin (1E31).

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-hydroxy flavone 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-hydroxy flavone was never reported previously. To develop compounds with anti-cancer and other pharmacological properties from natural substances has become a focus of interest[45]. 7-hydroxylflavone 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[13], inhibition of fatty acid synthesis, topoisomerase inhibition[14], induction of cell cycle arres, accumulation of p53[15] or enhanced expression of c-fos and c-myc[16] . 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-hydroxyflavone is an active anti-cancer compounds which work through apoptosis induction. These findings may be useful for developing potent anti-cancer compounds from flavonoids for potential clinical applications.

MOLECULAR INTERACTIONS OF 7-HYDROXY FLAVONE, 7,3',4'-TRIHYDROXYFLAVONE, 7,8,3'-TRIHYDROXYFLAVONE 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).

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

REFERENCES

1. Schnekenburger M, Dicato M and Diederich M: Plant-derived epigenetic modulators for cancer treatment and prevention. Biotechnol Adv 32: 1123-1132, 2014

2 DeVita VT Jr, Young RC and Canellos GP: Combination versus single agent chemotherapy: A review of the basis for selection of drug treatment of cancer. Cancer 35: 98-110, 1975.

3 Kühnau J: The Flavonoids. A Class of Semi-Essential Food Components: Their Role in Human Nutrition. In: World Review of Nutrition and Dietetics. Bourne GH (ed). Karger, Basel, pp117-191, 1976. .

4 Benbrook CM: Elevating Antioxidant Levels in Food through Organic Farming and Food Processing. An Organic Center, State of Science Review. The Organic Center for Education and Promotion, 2005.

5 Nikitovic D, Tsatsakis AM, Karamanos NK and Tzanakakis GN: The effects of genistein on the synthesis and distribution of glycosaminoglycans/proteoglycans by two osteosarcoma cell lines depends on tyrosine kinase and the estrogen receptor density. Anticancer Res 23

(1A): 459-464, 2003.

6 Lin X, Li X, Lin X. 2020. A review on applications of computational methods in drug screening and design. Molecules 25(6):1375. doi:10.3390/molecules25061375.

7 Deshpande RR, Tiwari AP, Nyayanit N, Modak M. 2020. In silico molecular docking analysis for repurposing therapeutics against multiple proteins from SARS¬CoV¬2. Eur J Pharmacol. 886:173430. doi:10.1016/j.ejphar.2020.173430.

8 Pratama MRF, Poerwono H, Siswodihardjo S. 2020. Molecular docking of novel 5¬O-benzoylpinostrobin derivatives as SARS¬CoV¬2 main protease inhibitors. Pharm Sci. 26(Suppl1):S63–S77. doi:10.34172/PS.2020.57.

9. Choi, S. I., Jeong, C. S., Cho, S. Y., and Lee, Y. S., Mechanism of apoptosis induced by apigenin in HepG2 human hepatoma cells: involvement of reactive oxygen species generated by NADPH oxidase. Arch. Pharm. Res., 30, 1328-1335 (2007).

10. Vijayababu, M. R., Kanagaraj, P., Arunkumar, A., Ilangovan, R., Dharmarajan, A., and Arunakaran, J., Quercetin induces p53-independent apoptosis in human prostate cancer cells by modulating Bcl-2-related proteins: a possible mediation by IGFBP-3. Oncol. Res., 16, 67-74 (2006)

11. Bast, A., Haenen, G. R., Bruynzeel, A. M., and Van-der-Vijgh, W. J., Protection by flavonoids against anthracycline cardiotoxicity: from chemistry to clinical trials. Cardiovasc. Toxicol., 7, 154-159 (2007).

12. Chen, D., Daniel, K. G., Chen, M. S., Kuhn, D. J., LandisPiwowar, K. R., and Dou, Q. P., Dietary flavonoids as proteasome inhibitors and apoptosis inducers in human leukemia cells. Biochem. Pharmacol., 69, 1421-1432 (2005).

13. Yoon, G., Kang, B. Y., and Cheon, S. H., Topoisomerase I inhibition and cytotoxicity of licochalcones A and E from Glycyrrhiza inflata. Arch. Pharm. Res., 30, 313-316 (2007)

14. Singh, R. P. and Agarwal, R., Natural flavonoids targeting deregulated cell cycle progression

in cancer cells. Curr. Drug. Targets., 7, 345-354 (2006).

15. Ganguly, C., Saha, P., Panda, C. K., and Das, S., Inhibition of growth, induction of apoptosis and alteration of gene expression by tea polyphenols in the highly metastatic human lung cancer cell line NCI-H460. Asian. Pac. J. Cancer. Prev., 6, 326-331 (2005).