INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 6.078
(Volume 6, Issue 3)
Available online at: www.ijariit.com

To investigate potential target based phytochemicals against COX 2 & STAT 3 protein of Squamous Cell Carcinoma of Head & Neck using In-Silico approach

Priyankal D. Sharma
priyankalsharma9@gmail.com
Guru Nanak Khalsa College of Arts, Science and Commerce (Autonomous), Mumbai, Maharashtra

Aparna Hiren Patil Kose
aparna.patil@gnkhalsa.edu.in
Guru Nanak Khalsa College of Arts, Science and Commerce (Autonomous), Mumbai, Maharashtra

Chintan Navnit Shah
chintan9086@gmail.com
Guru Nanak Khalsa College of Arts, Science and Commerce (Autonomous), Mumbai, Maharashtra

ABSTRACT

Cancer is not just one disease but many diseases affecting the world over. Carcinoma is a type of cancer arise from epithelial cells. Squamous-cell carcinoma of the head and neck (SCCHN) represents a heterogeneous disease entity, with various etiological factors implicated in the genesis of distinct molecular subsets of tumors, which exhibit different biological and clinical behavior. Historically, SCCHN has been about twice as common in men as in women and more frequent in people over 50 with growing incidence reported among younger people. Alcohol and tobacco are common etiologic factors for SCCHN, linked to at least 75% of cases. Systems biology emphasizes the need for molecular characterization of cancer development. Molecular tools are screened as means of candidate biomarkers leading to cancer. Overexpression of proteins causing tumorigenesis is better understood by studying their role in cancer proliferation. COX 2 and STAT3 are pathway-mediated bioagents in head and neck cancer considered as targetable protein. Phytochemicals such as Rutin, Quercetin and Ellagic acid are well-known inhibitors of both COX 2 and STAT3 proteins. In-silico approach was used to analyze binding interactions of phytochemicals against selected protein structures of COX 2 and STAT3 using docking studies thereby forming drug able agents. The main objective is to find an alternative treatment to chemoprevention therapy. This research work aims to carry out systematic review of head and neck cancer in order to develop potential target-based therapy. Therefore, Rutin against 5IKR, 5F19, Quercetin against 6NJS and Ellagic acid against 6QHD may be considered to be potential anti-cancer against SCCHN target which further needs to be explored for drug development process.

Keywords— *Squamous cell carcinoma of head and neck (SCCHN), COX 2, STAT 3, Phytochemicals and its analogues, Molecular docking*

1. INTRODUCTION

Cancer is a genetic disease that is, it is caused by changes to genes that control the way our cells function, especially how they grow and divide. Genetic changes that cause cancer can be inherited from our parents. They can also arise during a person's lifetime as a result of errors that occur as cells divide or because of damage to DNA caused by certain environmental exposures. The genetic changes that contribute to cancer tend to affect three main types of genes- proto-oncogenes, tumor suppressor genes, and DNA repair genes. These changes are sometimes called "drivers" of cancer. [1]

Carcinomas are the most common type of cancer. They are formed by epithelial cells, which are the cells that cover the inside and outside surfaces of the body. Squamous cell carcinoma of the head and neck (SCCHN) represents more than 90% of all head and neck cancers and arises from the mucosa of the upper aero digestive tract multiple anatomic sites. Approximately 600,000 new cases are diagnosed each year, including about 50,000 in the United States. HNSCC occurs most often in men in their 50s or 60s, although the incidence among younger individuals is increasing. The worldwide incidence of SCCHN exceeds half a million cases annually, making it the sixth most frequent cancer globally. Head and Neck carcinoma is a cancer that starts in the lip, oral cavity (mouth), nasal cavity (inside the nose), paranasal sinuses, pharynx and larynx. They originate from epithelium and spread to head and neck. [2]

This work focuses on the mechanisms that underlie fundamental processes such as cell growth, the transformation of normal cells to cancer cells, and the spread, or metastasis, of cancer cells. Knowledge gained from such studies deepens our understanding of cancer and produces insights that could lead to the development of new clinical interventions. The greatest advances in

© 2020, www.IJARIIT.com All Rights Reserved

understanding the origin and progression of cancer during the past decade have occurred in the field of molecular genetics. [3]

Signaling pathways propagate information from one part or sub-process of the cell to another, often via a series of protein covalent modifications, such as protein phosphorylation. Dysregulation of biological processes by aberrant signaling pathways causes cancer. Proteins related to SCCHN are reported and their mechanism understood by identifying signaling pathways that play a role in cancer proliferation. COX 2 and STAT3 are bioagents whose reaction mechanisms within cancer progression are subject to study. The two factors COX 2 and STAT3 are pathway-mediated targets. Over expressions of these factors causes tumorigenesis. The goal is to target these factors by inhibiting their growth and progression at molecular level thereby preventing tumor formation. This helps in early diagnosis and prevention. [4]

Complex signaling pathways of a biological system are understood using pathway database. In past years, comprehensive representations of cell signaling pathways have been developed by manual curation from literature. Mathematical modelling techniques are used to simulate the complex behavior of cell signaling networks, which ultimately sheds light on the mechanisms leading to complex diseases or helps in the identification of drug targets. KEGG is not only a database for pathways but consists of 19 highly interconnected databases, containing genomic, chemical and phenotypic information. KEGG categorizes its pathways into metabolic processes, genetic information processing, environmental information processing, including signaling pathways, cellular processes, information on human diseases and drug development. COX 2 and STAT3 pathway system was observed and their interactions studied. [5]

Over the years natural medicine cancer researchers around the world have developed highly effective natural cancer treatments which use a combination of tactics to slow down the spread of the cancer; safely and gently kill cancer cells; reverting cancer cells into normal cells. The term "chemoprevention" refers to efforts to prevent or delay the development of cancer by taking medicines, vitamins or other agents'. The ideal chemo preventive agent will not significantly alter quality of life, is inexpensive, safe, well tolerated, and effective. However, some chemo preventive drugs may have severe side effects in some patients, which is an issue when considering long-term administration of a compound to healthy people who may or may not develop cancer. Chemotherapy drugs are the most toxic substances ever put deliberately into the human body. They are known poisons; they are designed poisons. Effectiveness is only temporary. The effectiveness of chemotherapy is being rapidly overtaken by new targeted therapies. [6]

The idea that nutrition is an important factor in cancer causation is not new. Roger Williams in 1908 observed that excessive feeding especially meat, deficient exercise and probably lack of sufficient vegetable food are the predisposing factors for cancer. Role of diet therefore plays a special importance. The phytochemicals are under observation. Their role as inhibitors is reported. Various dietary sources contain these substances that act as cancer preventives and therapeutic agents. [7]

Target based therapy attack specific cancer cells yet leaves the rest of the body untouched. Phytochemicals can block the activity of these agents thereby preventing further reactions. The basic idea is to find an alternative means of treating head and neck cancer by developing potential therapeutics. In-silico methods give tremendous opportunity to design and develop potential drug targets. Structural analysis of molecules (proteins) is carried out using bioinformatics tools. [8]

Understanding the role of phytochemical is important in order to develop potential drugs against the target agents of SCCHN. Both protein (COX 2 and STAT3) and phytochemical compounds (*Rutin, Quercetin and Ellagic acid*) were considered for study. Bioinformatics study applies computational simulations and does predictions of biological components. Pathway databases provide huge information about the disease mechanism. COX 2 (51KR, 5F19) and STAT 3 (6NJS, 6QHD) protein structures were screened from PDB database and downloaded in PDB file format. Phytochemical screening was carried out using PubChem Compound database. Docking analysis of these complex structures were performed via Auto Dock Vina tool. Discovery Studio helped visualize docked structures. Inferences were drawn in accordance with the results obtained. This work focuses on *in silico* methods of developing potential therapeutics for target-based diagnosis and treatment of cancer. Therefore, these phytochemicals can be considered as potent medicine.

2. METHODOLOGY

2.1 Selection of proteins

Proteins related to SCCHN are reported and their mechanism understood by identifying signaling pathways that play a role in cancer proliferation. COX 2 and STAT3 are bioagents whose reaction mechanisms within cancer progression are subject to study. The two factors COX 2 and STAT3 are pathway-mediated targets. Over expressions of these factors causes tumorigenesis. The goal is to target these factors by inhibiting their growth and progression at molecular level thereby preventing tumor formation. The structures were downloaded from PDB database

2.2 Selection of ligands

Approximately 2500 medicinal plant species have been recorded globally [19,20] to treat a myriad of inflictions and diseases. Polyphenols, alkaloids, flavonoids, saponins, quinones, terpenes, proanthocyanins, lignins, tannins, polysaccharides, steroids, thiosulfonates and coumarins are prominent bioactive phytochemicals, which have been observed to combat infections. Many traditional medicinal plants and herbs have been reported. Total phytochemicals as ligands was selected on basis of their anticancer properties and their 3D conformer was download from PubChem database.

2.3 Docking studies

Proteins were docked with selected phytochemicals to find a potent natural molecule for SCCHN which acts as an anti- cancer agent by using AutoDock Vina software as it's an automated procedure for predicting the interaction of ligands with biomacromolecule targets.

© 2020, www.IJARIIT.com All Rights Reserved

2.4 Visualization

Interaction between ligands (phytochemicals) & proteins was visualized by Discovery Studio 4.1 Visualizer. Discovery Studio is a comprehensive software suite for analyzing and modelling molecular structures, sequences, protein-ligand interactions and other data of relevance to life science researcher

3. RESULTS

3.1 Docking results

Molecular docking approach was used to determine potent phytochemicals which will show the best binding energy with the selected proteins. Total 6 ligands (namely Rutin, quercetin, ellagic acid, myricetin, Kaempferol, apigenin) were docked against each of 4 proteins (namely 5IKR, 5F19, 6NJS, 6QHD) using AutoDock tool where coordinates for each docked conformation to the docking log file is been created, along with information on clustering and interaction energies and provides options for analyzing the information stored in the docking log file. With this study; it was found that docking score with all 4 proteins were showing the highest binding affinity as binding energy obtained in negative values indicates that the scores obtained are highly efficient. The following table shows top docking poses ranked according to their binding energy.

Table 1: Ranked Docking conformations showing binding energy of docked structures

Sr. No.	PDB ID	Protein	Name of Phytochemicals	Binding Affinity (kcal/mol)	Binding residue
1	5IKR	COX 2	Rutin	-10.5	GLU236, LEU224, GLY225, ARG33, SER143, TRP139, LEU145, ARG376
2	5F19		Rutin	-9.6	ASP133, TYR134, PRO154, TYR136, ASP157, GLN327, TRP323, PRO156, ASN34
3	6NJS	STAT 3	Quercetin	-8.0	LYS370, ASP369, LEU438, HIS437, SER381, VAL490
4	6QHD		Ellagic acid	-7.5	LYS573, ASP570, THR515, ASP566, HIS332, MET331, ARG335

3.2 Visualization results

Since docking was performed by AutoDock software; protein-ligand interactions of the docked structure were interested to be observed and performed using Discovery Studio 4.1 Visualizer. Following figures shows protein-ligand interactions viewed in Discovery Studio 19.1 Visualizer where amino acid residues are labelled. Top-ranked phytochemical (Table 1) were selected to show interaction with the all 4 proteins.

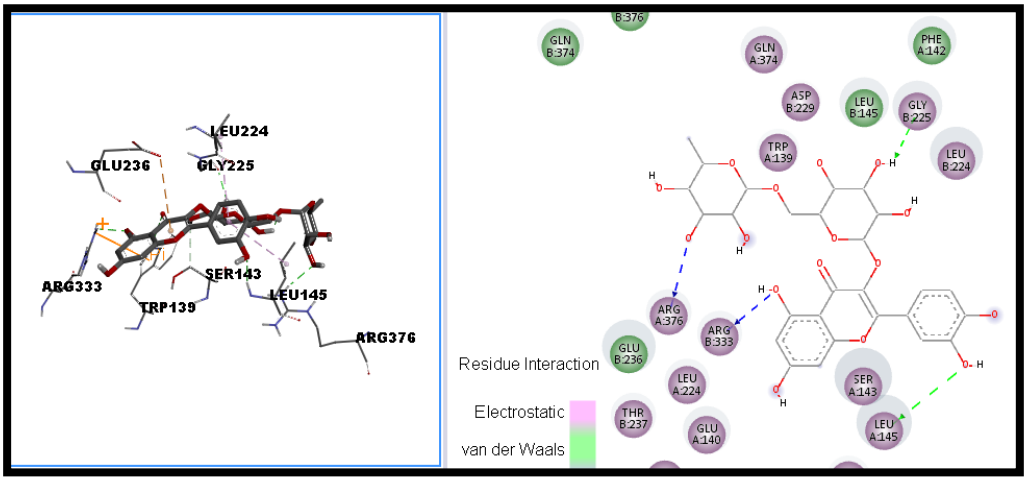

Fig. 1: 2D and 3D visualization of 5IKR docked with Rutin along with amino acid interaction

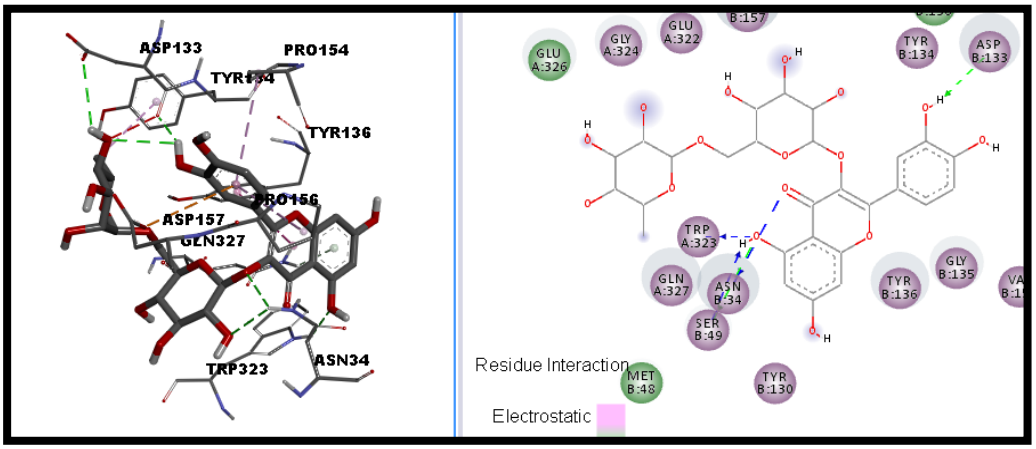

Fig. 2: 2D and 3D visualization of 5F19 docked with Rutin along with amino acid interaction

© 2020, www.IJARIIT.com All Rights Reserved

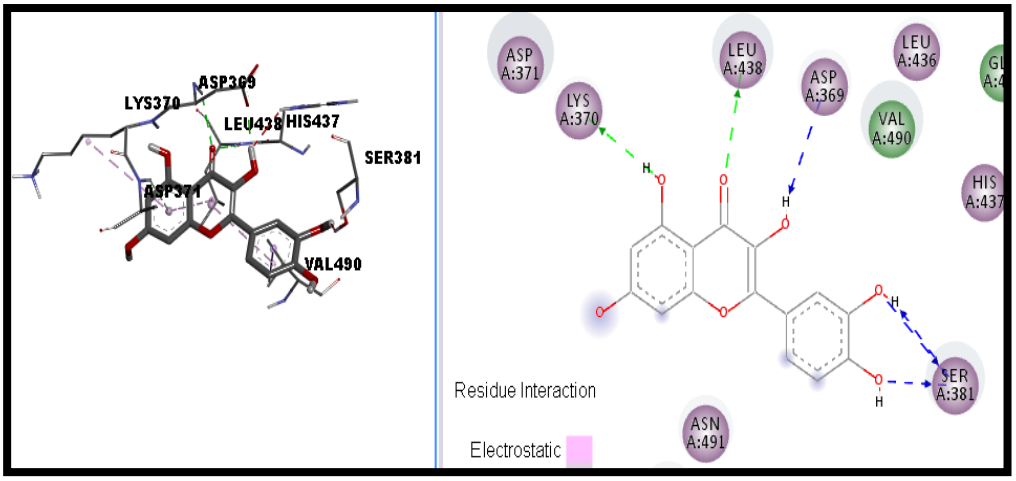

Fig. 3: 2D and 3D visualization of Quercetin along with amino acid interaction

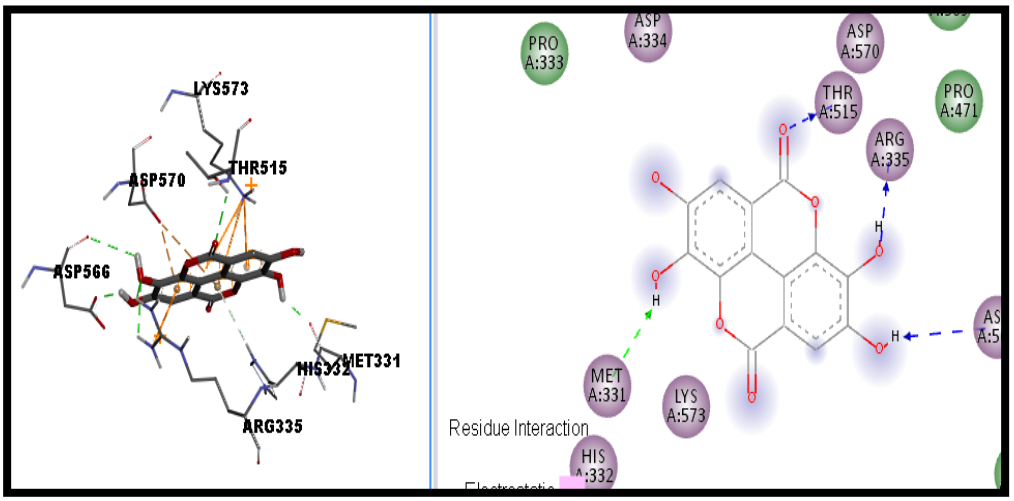

Fig. 4: 2D and 3D visualization of 6QHD docked with Ellagic acid along with amino acid interaction

3.3 Interpretation

Molecular docking is a computational method which aims to identify non-Covalent binding between protein (receptor) and a small molecule (ligand/Inhibitor). Docking predicts the mode of interaction between a target protein and a small ligand for an established binding site. Binding energy suggests the affinity of a specific ligand and strength by which a compound interacts with and binds to the pocket of a target protein. A compound with a lower binding energy is preferred as a possible drug candidate. In order to understand the effect of active phytochemicals compounds on COX 3 and STAT 3, molecular docking of 6 active phytochemicals compounds were selected after screening from literature

The phytochemical structures were retrieved from PubChem Compound Database based on their bioavailability. Rutin, Quercetin, Ellagic acid, Myricetin, Kaempferol, Apigenin are used as ligand molecules against COX 2 and STAT 3 protein and structures were subjected to geometry optimization i.e. protein and ligand optimization were done and further used for docking using AutoDock Vina tool.

Out of the 6 compounds, the best three namely Rutin (against 5IKR) exhibited the docked score (-10.5 Kcal/mol) with COX 2 protein, Quercetin (against 6NJS) exhibited the docked score (-8.5 Kcal/mol) and Ellagic acid (against 6QHD) exhibited the docked score (-7.5 Kcal/mol) with STAT 3 protein with the respect amino acid interaction (Table 1)

4. DISCUSSION

The majority of head and neck cancers (HNCs) around 90% arise in the squamous cells that line the moist mucosal surfaces of the oral cavity, pharynx, larynx, and paranasal sinuses and are referred to as squamous cell carcinoma of the head and neck (SCCHN). Cancer proliferation within SCCHN is better understood by molecular characterization of target protein. Proteins responsible for tumor growth are studied in accordance with the pathway in which they play a vital role. Pathway classification helps characterize biochemical reactions with respect to signaling factors that are responsible for cancer development. We have thus focused on targeting biological agents as means of screening candidate biomarkers for diagnosis of head and neck cancer.

At the same time phytochemicals are screened for their anticancer role because the objective is to develop potential drugs against the proteins in order to block or inhibit the activity of tumor. The small molecule knows as ligand usually fits within the protein's cavity which is predicted by the search algorithm and helps to predict the best binding affinity and actual active site of protein. Therefore, *in silico* approach i.e. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and cavity of the small molecule so that the interaction of two molecules can be determined and search the best orientation of ligand which would form a complex with overall minimum energy and prove as a

© 2020, www.IJARIIT.com All Rights Reserved

probable target site. Thus, leading to drug repurposing approach which would be the fast and most approapriate option to find therapeutic solutions for SCCHN.

5. CONCLUSION

The molecular docking analysis was performed using phytochemicals showing good binding affinity with four different HNSCC proteins responsible for it. The analysis was performed using Autodock tool, a freely available docking platform. All protein-ligand interactions were visualized using Discovery Studio 4.1 Visualizer, a free viewer for viewing and editing molecular structures.
Rutin showed binding affinity in the range of -10.5 to -9.5 kcal/mol. There are very few reported studies of Rutin docked with COX2 proteins and this study has discovered relatively better and promising binding affinities that may have huge impact in development of effective therapeutics against SCCHN patients. Thus, it is remarkable to observe the computational finding of novel and potent inhibitors for COX 2 and STAT 3.

Thus, very few in silico studies have been performed on head and neck squamous carcinoma with respect to phytochemicals. On the basis of RMSD value and negative scores and comparing current results with literature; we can conclude that the binding affinity obtained in the docking ranks are good. All the protein-ligand interactions of the docked structures showing amino acid residues assumed to be possible binding sites. This analysis can be further implemented in various related studies to suppress expression of COX2 and STAT3.

6. ACKNOWLEDGEMENT

The authors would like to show their gratitude to the GNIRD, Guru Nanak Khalsa College, the University of Mumbai and Dr. Kavita Pandey (Head, Department of Five Years Integrated Bioanalytical Sciences) for sharing their pearls of wisdom with us during the course of this research.

7. REFERENCES

[1] Jay D. Hunt, I. P. (n.d.). genetics and louisiana families. Retrieved from genetics and louisiana families: https://www.medschool.lsuhsc.edu/genetics_center/louisiana/article_cancer.htm
[2] NIH. (2017, march 29). National cancer institute. Retrieved from National cancer institute head and neck cancer: https://www.cancer.gov/types/head-and-neck/head-neck-fact-sheet
[3] Rashi Srivastava, 1. S. (2015). Identification of Ellagic acid analogues as potent inhibitor of protein Kinase CK2: A chemo preventive role in oral Cancer. Bioinformation.
[4] Chien-Yi Chan, c. a.-H.-F.-Y. (2016). Quercetin suppresses cellular migration and invasion in human head and neck squamous cell carcinoma (HNSCC). Biomedicine.
[5] Cancer research biology website: cancer.gov/research/areas/biology
[6] Hahn, W.C. and Weinberg, R.A. (2002) Modelling the molecular circuitry of cancer. Nat. Rev. Cancer 2 (5), 331–341.
[7] Anna Bauer-Mehren 1, Laura I Furlong, Ferran Sanz (2009). Pathway Databases and Tools for Their Exploitation: Benefits, Current Limitations and Challenges. Molecular system biology.
[8] Cancer tutor website: cancertutor.com
[9] About phytochemicals: phytochemicals.info/phytochemical
[10] Wu, C. H., Apweiler, R., Bairoch, A., Natale, D. A., Barker, W. C., Boeckmann, B., Suzek, B. (2006), The Universal Protein Resource (UniProt): an expanding universe of protein information. Nucleic Acids Research, 34(Database issue), D187–D191. doi: 10.1093/nar/gkj161
[11] About the PDB Archive and the RCSB PDB: https://www.rcsb.org/pdb/static.do?p=general_information/about_pdb/index.html
[12] Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Bryant, S. H. (2016), PubChem Substance and Compound databases, Nucleic Acids Research, 44 (Database issue), D1202–D1213, doi:/10.1093/nar/gkv951
[13] Protein structure refinement: https://dokhlab.med.psu.edu/chiron/login.php
[14] AutoDock Vina manual : http://vina.scripps.edu/manual.html
[15] Discovery Studio Visualizer: http://www.adrianomartinelli.it/Fondamenti/Tutorial_0.pdf
[16] Abdur Rauf 1, M. I.-U.-R. (2018). Anticancer Potential of Quercetin: A Comprehensive Review. Phytotherapy research.
[17] Chien-Yi Chan, c. a.-H.-F.-Y. (2016). Quercetin suppresses cellular migration and invasion in human head and neck squamous cell carcinoma (HNSCC). Biomedicine.
[18] Claudia Ceci, 1. P. (2018 Nov). Experimental Evidence of the Antitumor, Antimetastatic and Antiangiogenic Activity of Ellagic Acid. Nutrients.
[19] GUOJUN SUN, 1. S. (2019). Gallic acid as a selective anticancer agent that induces apoptosis in SMMC-7721 human hepatocellular carcinoma cells. Oncology letters.
[20] Muhammad Imran, 1. B.-R. (2019). Kaempferol: A Key Emphasis to Its Anticancer Potential. Molecules.
[21] Rashi Srivastava, 1. S. (2015). Identification of Ellagic acid analogues as potent inhibitor of protein Kinase CK2:A chemopreventive role in oral Cancer. Bioinformation.
[22] Xiaopeng Yan, Y. H. (September 2010). Rutin induces apoptosis via P53 up-regulation in human glioma CHME cells. Translational Cancer Research.

© 2020, www.IJARIIT.com All Rights Reserved