Original Research Article

Insilico Study of Anticolorectal cancer properties of some flavonoids and Terpenoids from African Propolis

Abstract:

Propolis, a resinous material produced by honey bees from plant exudates, has long been reported to been used in traditional herbal medicine and is widely consumed as a health aid and immune system booster. The colorectal cancer which is a world health emergency has renewed interest in propolis products worldwide; fortunately, various aspects of the Poly (ADP-ribose) polymerases (PARPs) mechanism are potential targets for propolis compounds. The treatment of CRC has been focus on the tumor site and stage of the disease using chemotherapy or radiotherapy, surgery, hormonal therapy, immunotherapy etc. Apoptosis have been used in preventing damaged cells from developing out but due to secondary mutations in apoptosis-regulating gene, it can distort this order. This work aimed at evaluating the anticancer potential of some flavonoids and terpenoids from African propolis which can inhibit the protein of PARPs therefore preventing the growth of the cancer cell. From the result β- amyrin and naringin showed the best binding affinity and fit at -11.9 kcal/mol and -11.7 kcal/mol respectively which was better than standard drugs Irinotecan -11.2 kcal/mol and Doxorubicin -9.1 kcal/mol with cocrystalline ligand at -11.2 kcal/mol. Other compounds also showed very high binding affinity of more than -9.0 kcal/mol suggesting the propolis compounds as potential anticancer compounds.

Keywords: Molecular docking; Anticolorectal cancer; propolis; Poly (ADP-ribose) polymerases (PARPs) ; Flavonoids; Terpenoids,

Introduction:

Cancer is a genetic disease that is said to be multifactorial and also one of the diseases caused by uncontrolled proliferation of abnormal cells division in the body [1]. In comparison with other diseases cancer is complex in nature and therefore has many potential molecular targets for therapeutics development [2.] Cancer have remained a global health challenge as there are over 200 types of cancer mostly named after the tissue they were found in for the first time like colorectal cancer, breast cancer, skin cancer, lungs cancer, bone cancer etc. Cancer has been reported as one of the significant causes of death in the 21st century [3.] World Health Organization (WHO) reported cancer in 2015 as the second leading causes of death of people below 70 years in 91 different countries and the global increase of 18.1 million new cancer cases and 9.6 million cancer related deaths have been reported by Bray et al [4.] Colorectal cancer (CRC) which is one of the prominent cancer have been reported as the third highest prevalence rate of all cancer in the world and it cases are estimated to reach 2.4 lakhs by 2035 [5-6]. The treatment of CRC has

been focus on the tumor site and stage of the disease using chemotherapy or radiotherapy, surgery, hormonal therapy, immunotherapy etc. [7.] Apoptosis have been used in preventing damaged cells from developing out but due to secondary mutations in apoptosis-regulating gene, it can distort this order [8.] Natural products have been the bedrock of modern therapeutic medicine as most of drugs have their source from natural products, either as dietary supplement or synthetic analogues [9.] Most flavonoids and other phytochemicals found in nature has been reported to trigger endoplasmic reticulum stress induces tissue damage through apoptosis and necrosis, [10-11] modification of some bioactive compounds have been implored to improve bioavailability, specificity and therapeutic effectiveness as well as other variety features which includes implementation of potential chemotherapeutic agents [12-14.] Propolis which is one of the natural products from bees have been acclaimed and reported as a medicinal product, which is a complex resinous product having many compositions of phytochemicals which can change depending on collection site, botanical origin, climate condition, trees around and extraction methods. Propolis has been used ethnomedically in ancient times as a remedy for varieties of disease and recently interest has been renewed in reinvestigating the drug potentials. It has been reported to have antioxidant and antitumor properties [15.] Flavonoids which are the most common phytochemicals found in propolis have been reported as phytochemicals having highest antioxidant, antitumor, cytotoxic and chemopreventive properties [16.] Some of the flavonoids that have been isolated from African propolis include Acacetin-Algeria, Quercetin-Algeria, Pinocembrin-Algeria and Egypt, Naringenin-Algeria, Chrysin-Algeria and Egypt, Apigenin-Algeria, Kaempferol-Algeria, Macarangin-Kenya and Nigeria, Liquiritigen-Nigeria, Narangin-.etc. [17.]

Poly (ADP-ribose) polymerases (PARPs) activate DNA repair mechanisms upon stress and cytotoxin-induced DNA damage and inhibition of PARP activity is a lead in cancer drug therapy[18.] PARPs-1 functions as a DNA damage sensor and signaling molecule. When it binds with DNA, the activated PARP cleaves NAD(+) into nicotinamide and ADP-ribose and polymerizes the ADP-ribose to nuclear acceptor proteins like histones, PARP itself and transcription factors which contribute to inflammatory signal transduction processes. Activation of PARP has been connected in the pathogenesis of stroke and other diseases. Inhibition of PARP by pharmacological agents has proved useful for the therapy of cancer [19.] Colorectal cancer is common to both men and women, in terms of morbidity is the third most common cancer, in terms of mortality is rated second. About 10% cases of cancer in the world is colorectal cancer and drugs like cetuximab, Deracizumab and camptosar have been used in the management of colorectal cancer but their effects vary from patient to patient, and the drugs have been reported to have some side effect on the patients. Therefore the search for natural alternative remains sacrosanct [20] with the help of molecular docking which have been used in computer aided drug design as a vital tool. However this work will focus on the use of phytochemical compounds (Flavonoids and Terpenoids) from African Propolis in docking on a protein from PARP to know whether it can inhibit its action and also to identify the active site,

binding affinity, ligand protein interaction and compare to the result of the docking of standard cancer drugs and the co-crystallized ligand.

Material and Methods

Protein receptors and ligand retrieval and preparations

The list of some flavonoids and terpenoids from African propolis with proven anti-cancer properties were retrieved from literature [17.] Three dimensional (3D) structures of the drugs, flavonoid and terpenoid compounds were retrieved from PubChem web server in simple document format (SDF). They were optimized using Open babel in Python Prescription (version 0.8) which converted the ligands energetically to the most stable structures using Merk Molecular Force Field 94 (MMFF94). Similarly, the 3D X-ray crystallographic structure of the Poly (ADP-ribose) polymerases (PARPs) was retrieved from the RCSB protein data bank (PDB) (https://www.rcsb.org/) with ID 1UK0. The proteins were then prepared for docking and minimized using the relevant tools in Discovery studio

Molecular Docking

Prior to molecular docking analysis, proteins were pre-processed using Discovery Studio 2020. This step includes the removal of any hetero-groups, other chains and water molecules. The active site of the protein was identified using Discovery studio. Furthermore, the preparation of ligands and receptors in the PDBQT file format were carried out in the AutoDock tool. The molecular docking was carried out using AutoDock Vina to understand the interaction between receptors and ligands. A rigid-flexible docking was performed after setting a grid box surrounding the binding sites of the receptors at exhaustiveness = 8, center x = 5.62, center y = -0.97, center z = 32.52, size x = 20.61, size y = 23.59, size z = 23.74.

Result and Discussion

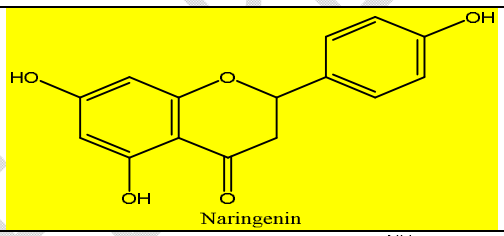
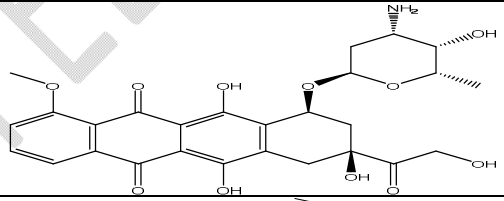
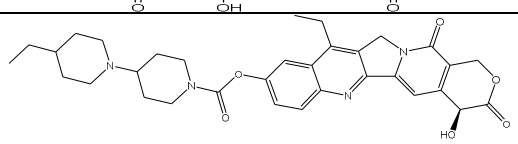
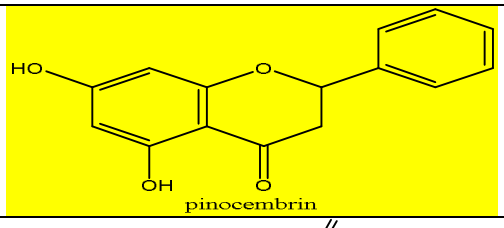
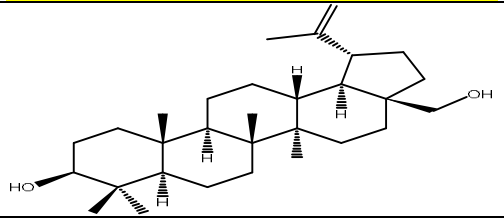
The result of the molecular docking of some flavonoids, terpenoids, drugs and the cocrystallized ligand on the protein of PARP are shown below below in table 1

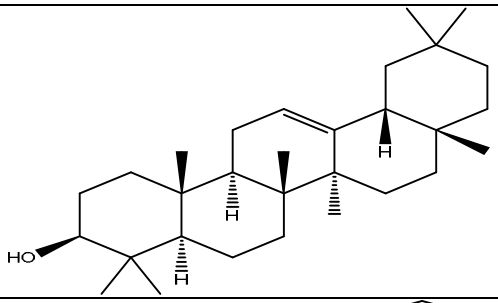
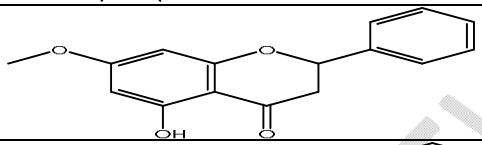
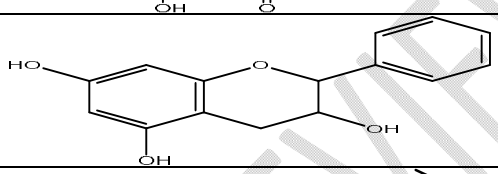
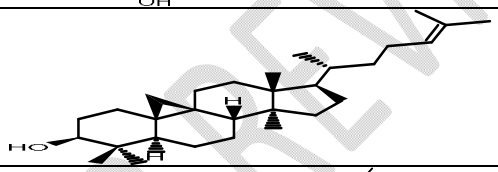
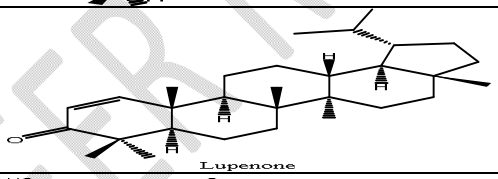
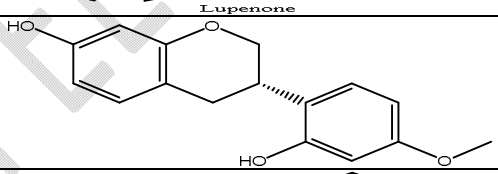
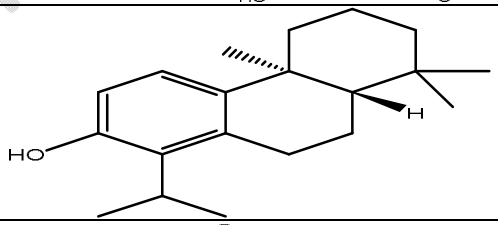
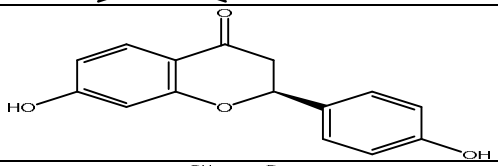
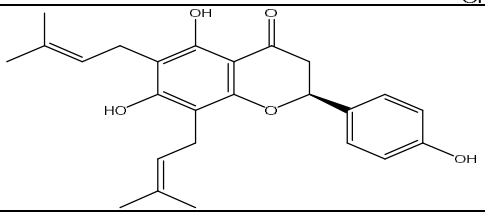
Fig 1: Protein of the cancer

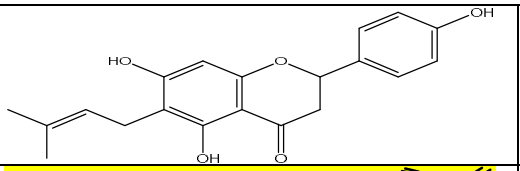
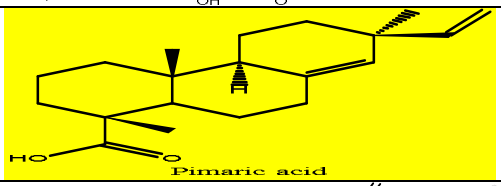
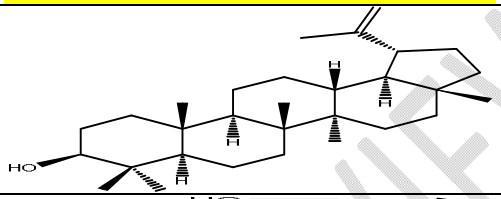
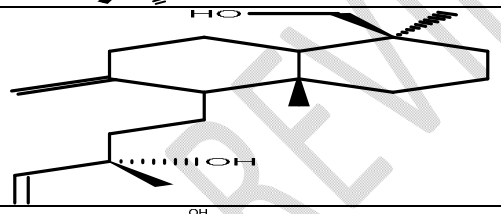
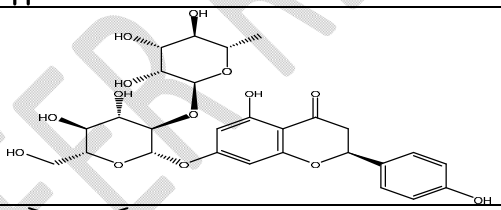
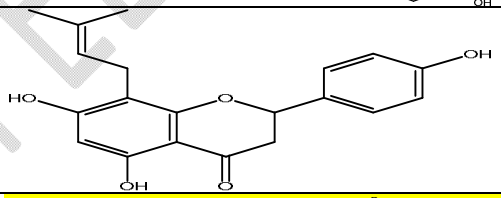
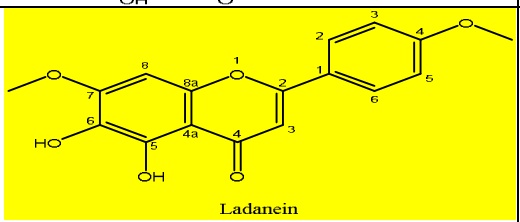
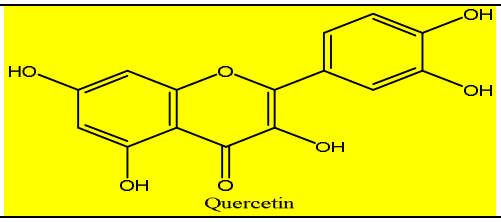
Picture of prepared protein Picture of Raw protein

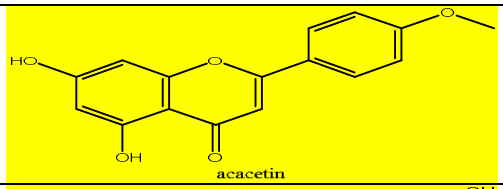
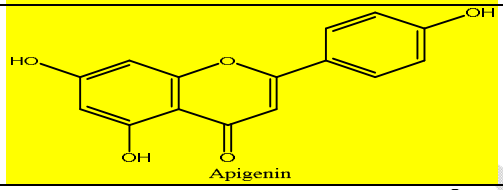
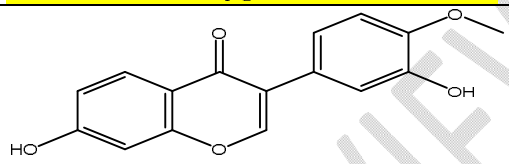
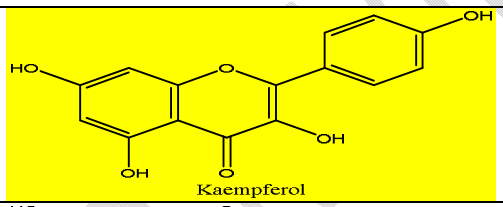
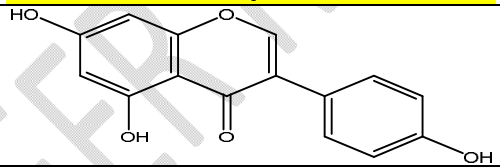
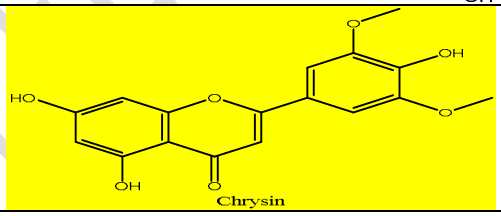
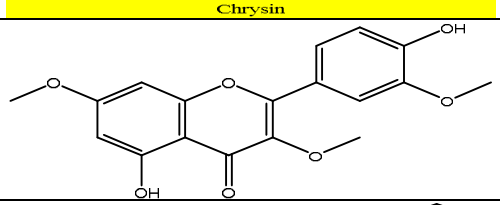
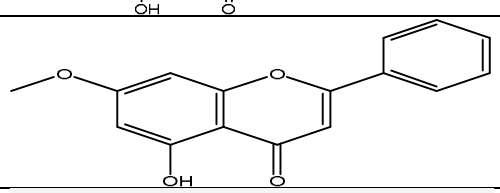
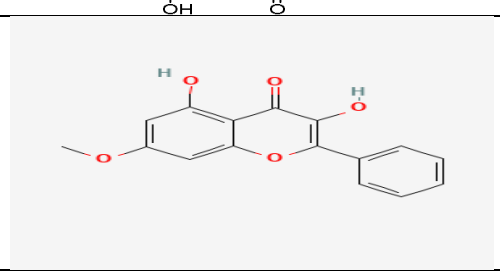
Docking Result:

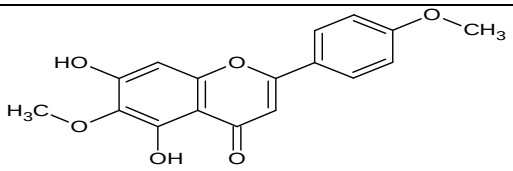
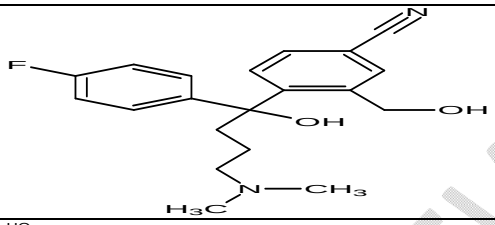
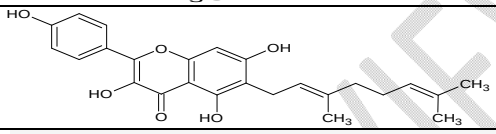
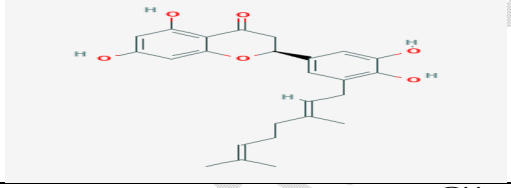
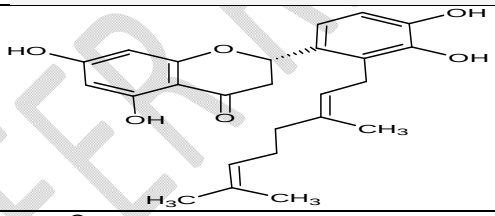
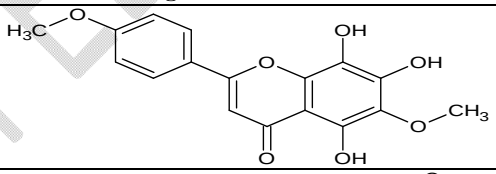
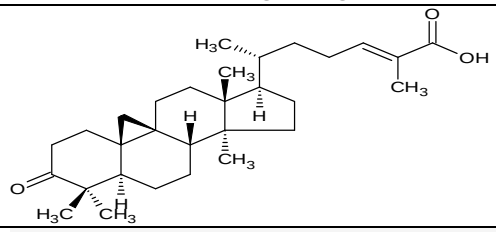
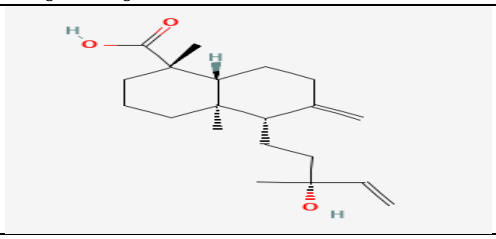
Table 1: The molecular docking result of the compounds, ligand and drugs.

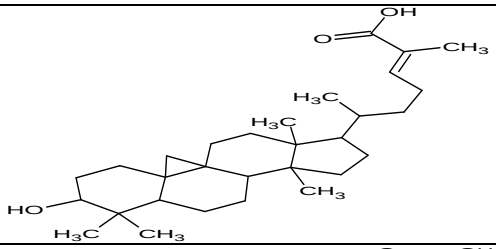
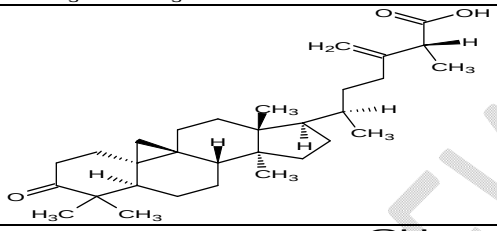
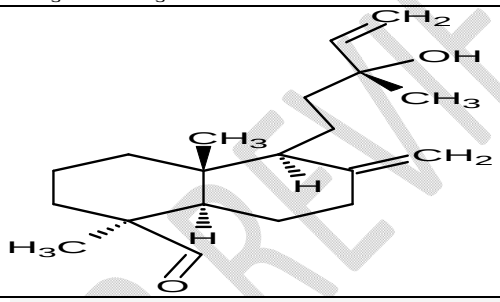
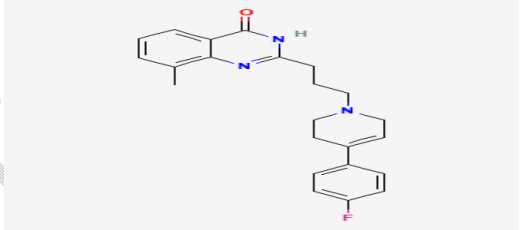
NAME	Pub CID	Structure	Binding affinity
Naringenin	932		-9.2
Drug(Doxorubicin)	31703		-9.1
Drug(Irinotecan)	60838		-11.2
Pinocembrin	68071		-8.9
Betulin	72326		-9.7

β-Amyrin	73145		-11.9
Pinostrobin	73201		-8.8
Pinobanksin	73202		-8.9
Cycloartenol	92110		-11
Lupenone	92158		-10.4
Vestitol	92503		-8.8
Totarol	92783		-10.2
Liquiritigenin	114829		-9.3
Lonchocarpol A	124035		-10.7

6-prenylnaringenin	155094		-9.9
Pimaric acid	220338		-8.9
Lupeol	259846		-10
Torulosol	349315		-7.7
Naringin	442428		-11.7
8-prenylnaringenin	480764		-9.6
Ladanein	3084066		-9.4
Quercetin	5280343		-9.5

Acacetin	5280442		-9.1
Apigenin	5280443		-9
Calycosin	5280448		-9.4
Kaempferol	5280863		-9
Genistein	5280961		-9.4
Chrysin	5281607		-8.9
Pachypodol	5281677		-8.7
Tectrochrysin	5281954		-8.9
Izalpinin	5318691		-8.7

Pectolinarigenin	5320438		-9.1
Citadiol	7472055		-8.3
Macarangin	10047854		-10.3
Isonymphaeol B	10070991		-11
Nymphaeol B	10387631		-10.2
Pilosin	12085264		-9.2
Mangiferonic acid	14034474		-11.1
Cupressic acid	44584269		-8.7

Mangiferolic acid	45270099		-11
Ambonic acid	101286242		-11.2
Torulosal	101306776		-8.1
Co-Ligand 2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2h)-pyridinyl]propyl}-8-methyl-4(3h)-quinazolinone	135460986		-11.6

The binding energy of some compounds isolated from the African propolis were shown in table 1 which were flavonoids and terpenoids other groups of phytochemicals were not analyzed for, because flavonoids and some terpenoids have been reported to have antioxidant and anticancer properties. The binding affinity score showed that all the compounds have high activity against the cancer protein and some of the compounds have activities higher than that of the control drugs and cocrystallized ligand. The control drug Irinotecan had -11.2 binding activity while Doxorubicin had binding affinity of -9.1. The cocrystallized ligand had binding affinity of -11.6, while most of the compounds have binding activities of -9 and beyond. The most active compound was β- amyrin with binding energy of -11.9 followed by naringin -11.7, ambonic acid -11.2, mangiferonic and mangiferolic acids with binding affinity of -11.1 and -11 respectively. Isonympeol B and cycloartenol have -11 which were all higher than the drug Doxorubicin -9.1. Other compounds were also higher with negative binding energy higher than -9 which showed that most of the compounds have very high activities over the cancer disease. The protein ligand interaction of the compounds with higher activities and that of the cocrystallized ligand and drugs are shown in Fig 2

Protein Ligand interaction

The figures below show the interaction of all the cocrystallized ligand, drugs and some phytocompounds that have higher activity with the protein.

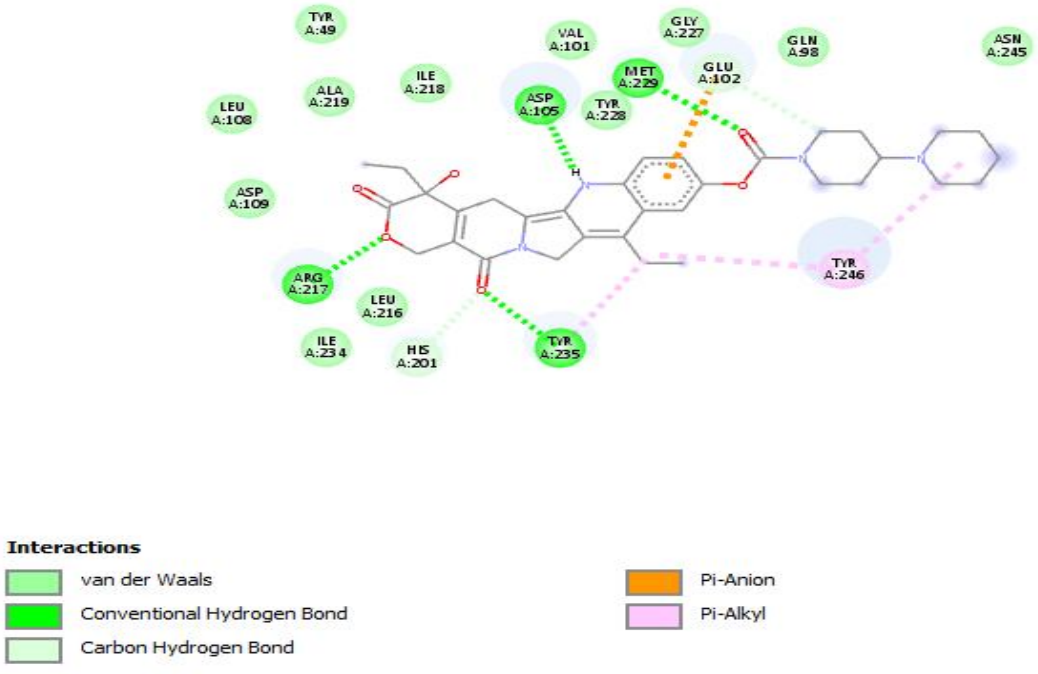


Figure 2.1: the interaction of the drug Irinotecan with the protein

From the drug and protein interaction shown above there was a conventional hydrogen bond interaction of ASP 105, ARG 217, MET 229 and TYR 235 while Carbon hydrogen bond interaction at TYR 49, GLN 98, VAL 101, LEU 108, ASP 109, LEU 216, ILE 218, ALA 219, GLY 227, ILE 234 and ASN 245. There were other interactions with weak bonds of van der waals, pi-anion and pi-alkyl.

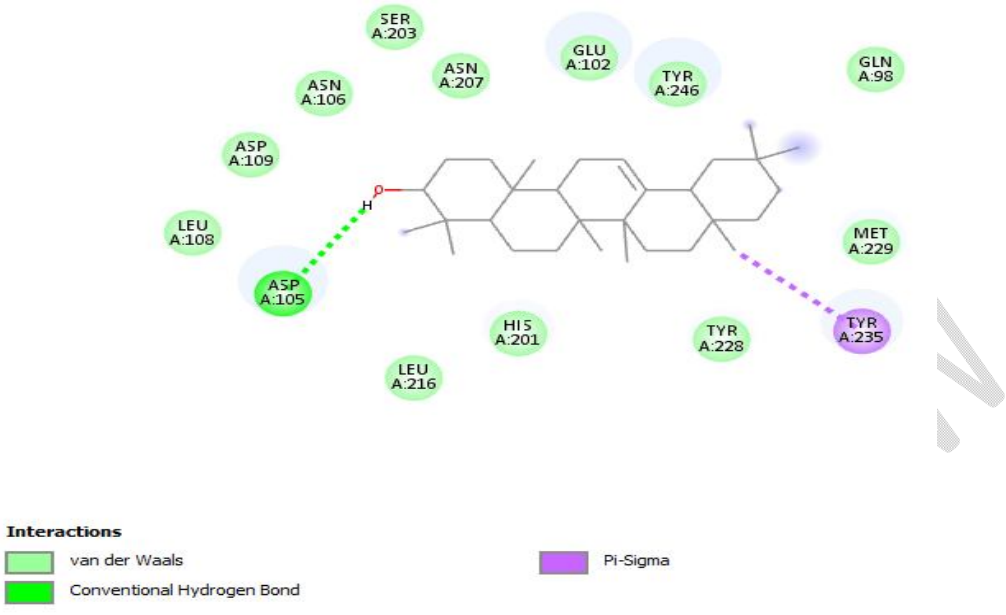


Figure 2.2: interaction of the protein with β-Amyrin

The β - amyrin and protein interaction are shown below there was a conventional hydrogen bond interaction of ASP 105, while Van der waals interaction at GLN 98, GLU 102, ASN 106, LEU 108, ASP 109, HIS 201, SER 203, ASN 207, LEU 216, TYR 228, TYR 246. There were other interactions with weak bonds of van der waals, pi-anion and pi-alkyl.

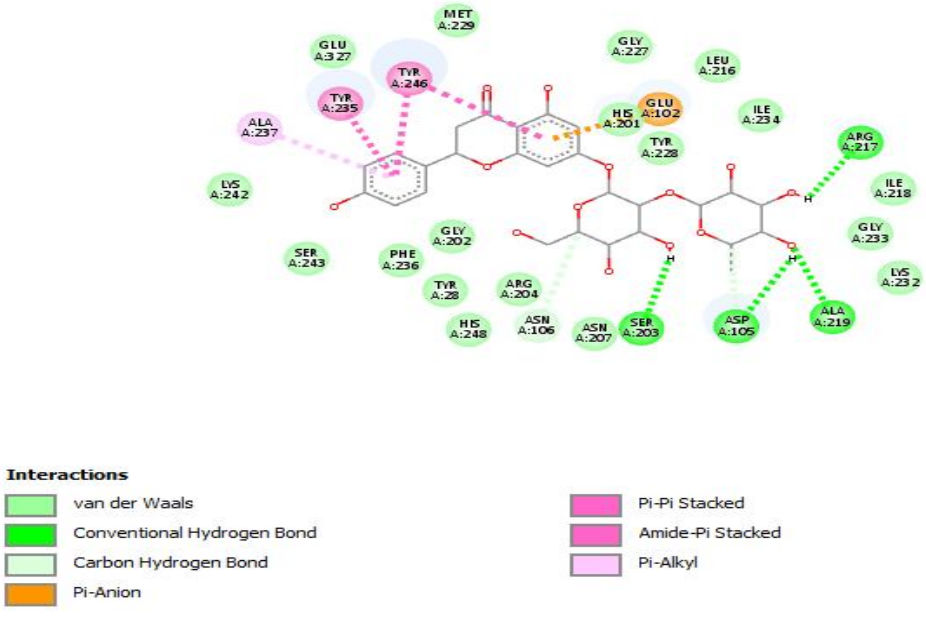


Fig 2.3: Interaction of the protein with Naringin

From the result there was a conventional Hydrogen bond with the amino acid of ASP 105, ARG 217, SER 203, ALA 219.

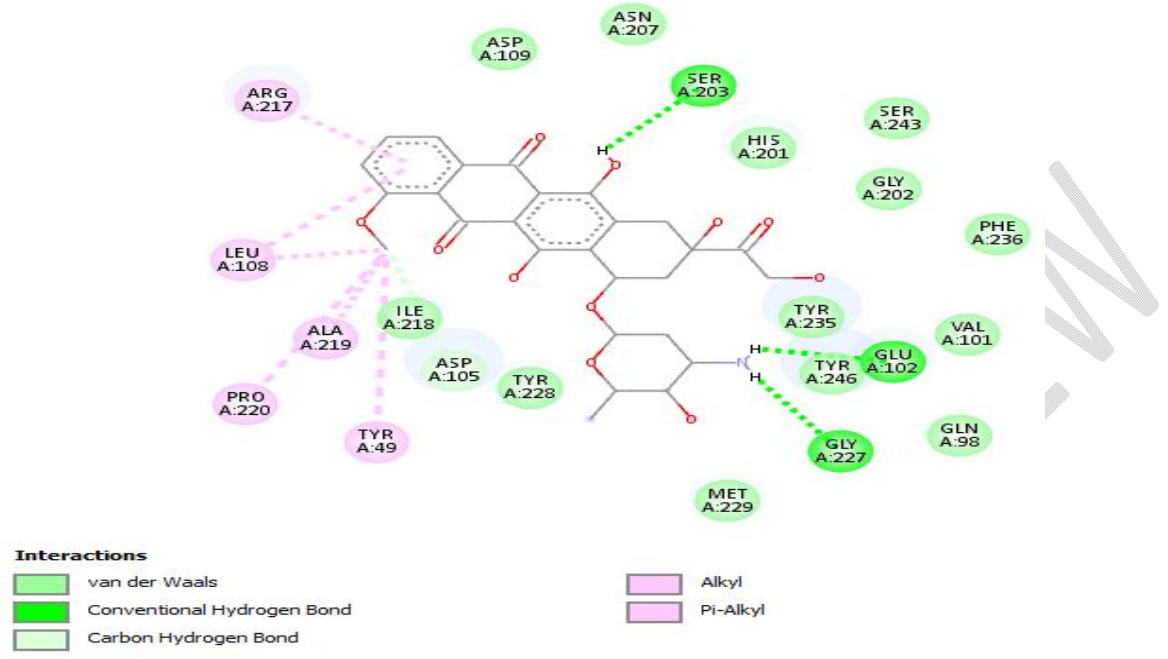


Fig 2.4: Interaction of the protein with Doxorubicin

From the result there was binding interaction of conventional hydrogen bond at SER 203, GLY 227, GLU 102 and other bond interaction which include carbon hydrogen bond, van der waal, Pi-akyl, and others at same binding site and amino acid.

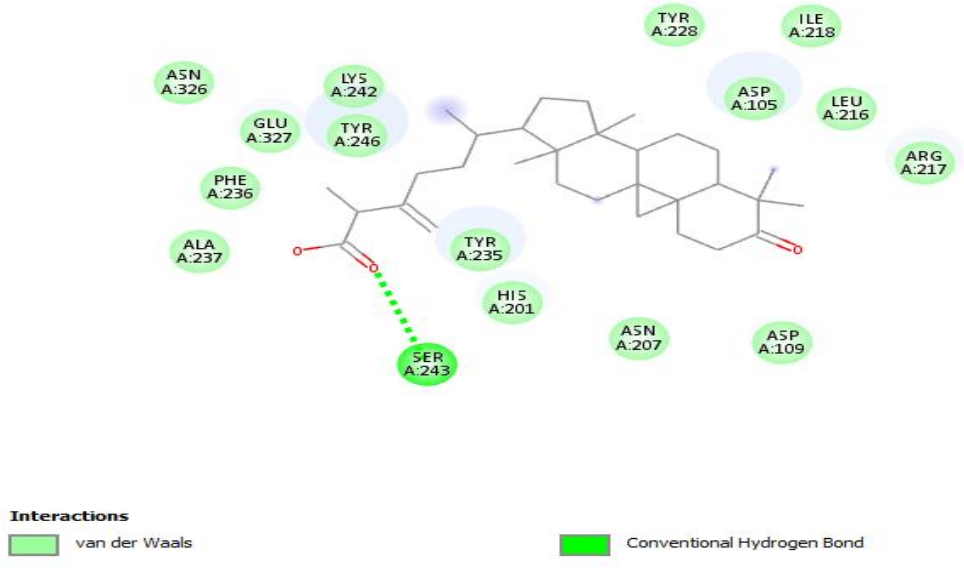
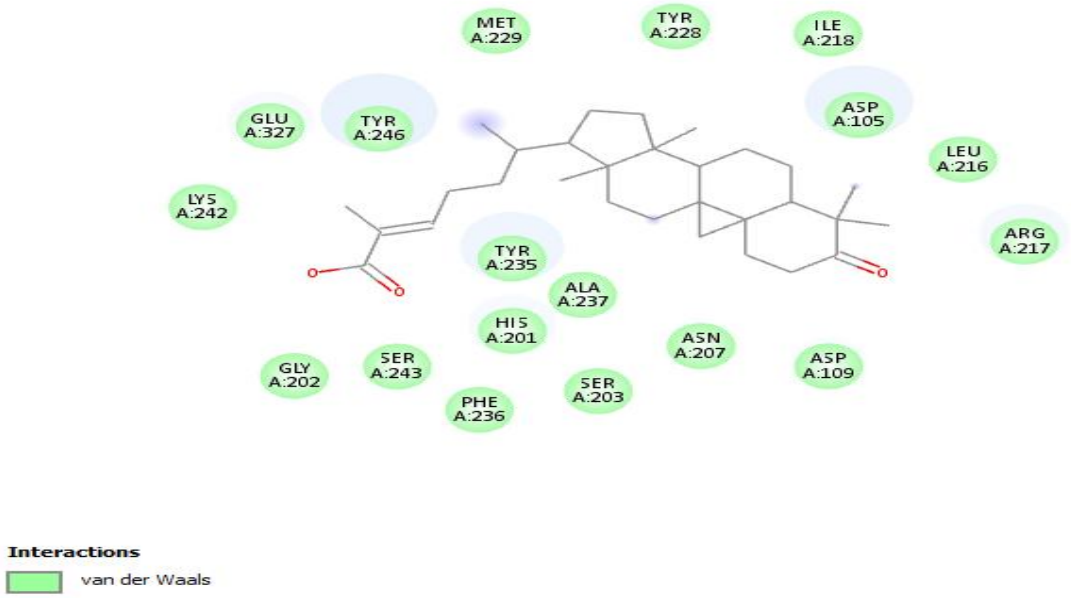
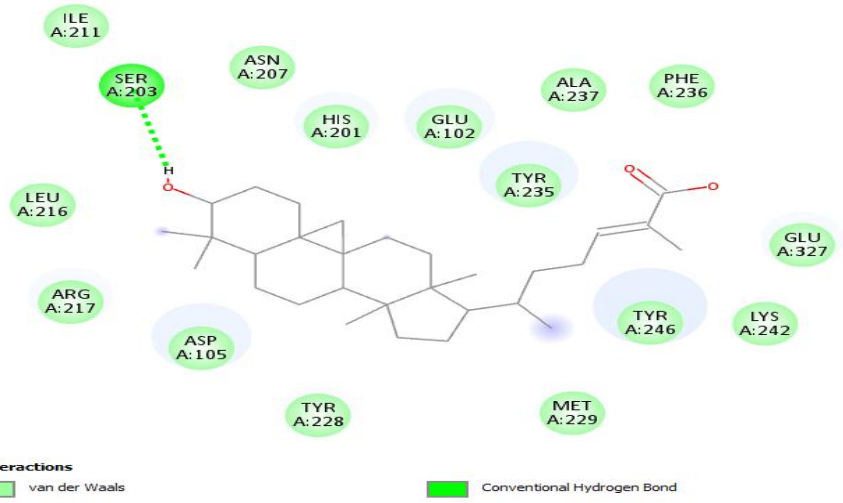


Fig 2.5: Protein interaction with Ambonic acid



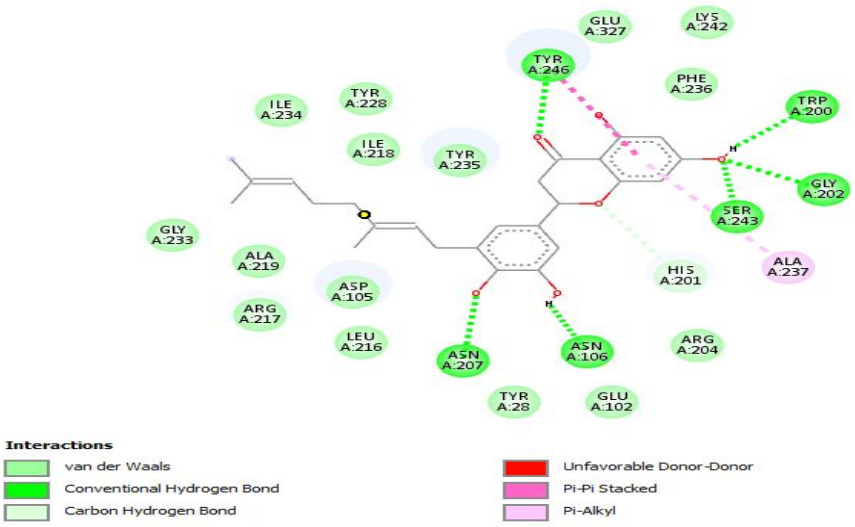
156

157 Fig 2.6: interaction with Mangiferonic acid



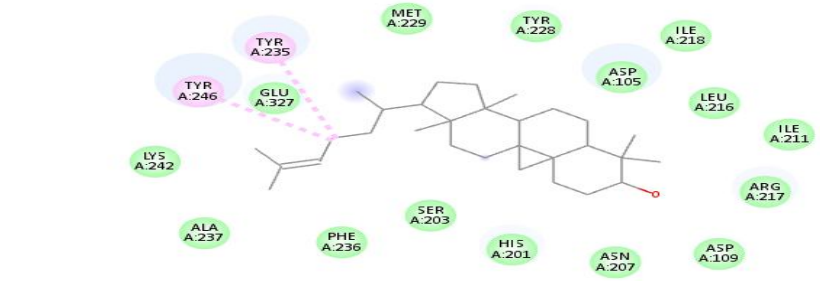
158

159 Fig 2.7: interaction with Mangniferolic acid



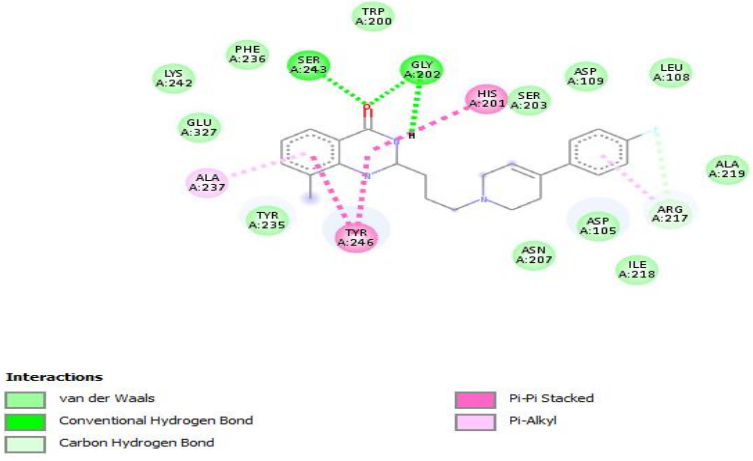
160

161 Fig 2.8: Interaction with Isonympeol A



162

163 Fig 2.9: interaction with Cycloartenol



164

Fig 2.10: Cocrystallizied Ligand interaction with the protein

From the result and interaction it showed that the compounds from propolis bind very well with the protein and also fit perfectly to the protein binding cavity. Most of the compounds have good binding affinity of more than -9 kcal/mol and the amino acids that the binding site from the cocrystalline ligand was also the amino acids that were also binded by the compounds and drugs thereby showing that the docking was at the binding site.

Conclusion

~~Honeybee~~ and propolis include a wide range of flavonoids and terpenoids compounds with several biological activities. The presented study screened *in silico* anticancer activities of some flavonoids and terpenoids from African propolis. The study revealed that some of the compounds have strong binding affinity and may inhibit the PARPs development therefore preventing cancer cell growth.
.

References

1. Sun, Y.S., Zhao, Z., Yang, Z.N., Xu, F., Lu, H.J., Zhu, Z.Y., Shi, W., Jiang, J., Yao, P.P., Zhu, H.P., 2017. Risk factors and preventions of breast cancer. International Journal of Biological Sciences 13 (11), 1387–1397. **https://doi.org/10.7150/ijbs.21635**.
2. Cui, W., Aouidate, A., Wang, S., Yu, Q., Li, Y., Yuan, S., 2020. Discovering Anti-Cancer Drugs via Computational Methods. Frontiers in Pharmacology 11, 733. https://doi. org/10.3389/fphar.2020.00733.
3. Yan, B., Yang, W. J., Han, X. Y., and Han, L. H. (2019). Crystal structures and antitumor activity evaluation against gastric carcinoma of two novel coordination polymers. Main Group Chem. 18, 239–246. doi: 10.3233/MGC-180748
4. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-a Cancer J. Clin. 68, 394–424. doi: 10.3322/caac.21492
5. Siegel,RL., Miller, K.D., Fuchs, H.E., Jemal, A. Cancer statistics, 2021, CA Cancer J. Clin. 71 (2021) 7–33, **https://doi.org/10.3322/caac.21654**.
6. Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA Cancer J. Clin. (2021) 1–41, **https://doi.org/10.3322/caac.21660**.
7. Hagan, T.L. Donovan, H.S. Self-advocacy and cancer: a concept analysis, J. Adv. Nurs. 69 (10) (2013) 2348–2359, **https://doi.org/10.1111/jan.12084**.

8. Zhang, X., Li, K., Feng, J., Liu, G., Feng, Y. Blocking the IGF2BP1-promoted glucose metabolism of colon cancer cells via direct de-stabilizing mRNA of the LDHA enhances anticancer effects, Mol. Ther. Nucleic Acids (2021), **https://doi.org/10.1016/j.omtn.2020.12.020**
9. Parmar, F., Patel, C., Highland, H., Pandya, H., George, L.B. Antiproliferative efficacy of kaempferol on cultured daudi cells: an in silico and in vitro study, Adv. Biol. 2016 (2016) 1–10, **https://doi.org/10.1155/2016/9521756**.
10. Sharma, V., Janmeda, P. Extraction, isolation and identification of flavonoid from Euphorbia neriifolia leaves, Arab. J. Chem. 10 (4) (2017) 509–514, **https://doi.org/10.1016/j. arabjc.2014.08.019**.
11. Liu, H. et al, The natural occurring compounds targeting endoplasmic reticulum stress, Evid. Based Complement. Alternat. Med. 2016 (2016), **https://doi.org/10.1155/2016/7831282 7831282**.
12. Patridge, E., Gareiss, P., Kinch, M.S., Hoyer, D. An analysis of FDA-approved drugs: natural products and their derivatives, Drug Discov Today. 21 (2) (2016) 204–207, **https://doi.org/** 10.1016/j.drudis.2015.01.009.
13. Newman, D.J., Cragg, G.M. Natural products as sources of new drugs over the 30 years from 1981 to 2010, J. Nat. Prod. 75 (3) (2012) 311–335, **https://doi.org/10.1021/np200906s**.
14. Mishra, B.B., Tiwari, V.K. Natural products: an evolving role in future drug discovery, Eur. J. Med. Chem. 46 (10) (2011) 4769–4807, **https://doi.org/10.1016/j.ejmech.2011.07.057**.
15. Ugariogu SN, Duru IA, Onwumere FC, Igoli JO. Physicochemical Assessment and Drug Potential of Some Phenylpropanoid and Flavonoid Compounds of Ethyl Acetate Eluate from Umudike Propolis. Trop J Nat Prod Res. 2020; 4(12):1208-1214. doi.org/10.26538/tjnpr/v4i12.30
16. Ugariogu SN, et al. Preliminary Pharmaceutical Active Ingredient and Micronutrient Evaluation of the Leaf of *Corchorus olitorius* (Ahiahara). Nat Ayurvedic Med 2020, 4(2): 000233.
17. Blicharska N. and Seidel V. Chemical Diversity and Biological Activity of African Propolis © Springer Nature Switzerland AG 2019 A. D. Kinghorn, H. Falk, S. Gibbons, J. Kobayashi, Y. Asakawa, J.-K. Liu (eds.) Progress in the Chemistry of Organic Natural Products, Vol. 109, **https://doi.org/10.1007/978-3-030-12858-6_3**

18. Lehtio L. et al. Structural basis for inhibitor specificity in human poly (ADP-ribose) polymerase-3 J Med Chem 2009; 52 (9):3108-3111
19. Southan G.J. and Szabo C Poly(ADP-Ribose) Polymerase inhibitors Current Medicinal Chemistry 2003; 10 (4) 321-340
20. Ikwu, F.A., Isyaku, Y., Obadawo, B.S, Lawal, H.A and Ajibowu, S.A Insilico design and molecular docking study of CDK-2 inhibitors with potent cytotoxic activity against HCT

116 colorectal cancer cell line. Journal of Genetic Engineering and Biotechnology 2020; 18 (51)1-12.