*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 focused on the tumor site and stage of the disease using chemotherapy or radiotherapy, surgery, hormonal therapy, immunotherapy etc. Although Aapoptosis is being 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 has remained a global health challenge as there are over 200 types of cancer, majority of them mostly were named after the tissue they were found to be infected 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]. In 2015, World Health Organization (WHO) reported cancer in 2015 as the second leading causes of death of in people below 70 years of age in 91 different countries, and the Bray et al, (year) reported a global increase of 18.1 million new cancer cases and 9.6 million cancer related deaths have been reported by Bray et al [4]. The prevalence rate of Colorectal cancer (CRC) which is recorded one of the prominent cancer have been reported as the third highest

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~~prevalence rate~~ of all cancers in the world. ~~and it~~ By 2035 the cases are estimated to reach over 2.4 lakhs ~~by 2035~~ [5-6]. The treatment of CRC ~~has been~~ focuses on the tumor site and stage of the disease using chemotherapy or radiotherapy, surgery, hormonal therapy, immunotherapy etc. [7]. Apoptosis ~~have been~~ is used in preventing damaged cells from developing out but sometimes 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 the drugs have their source from natural products, either as dietary supplement or synthetic analogues [9]. Most of the flavonoids and other phytochemicals found in nature ~~has been~~ are reported to trigger endoplasmic reticulum stress that may induce~~s~~ tissue damage through apoptosis and necrosis~~,~~ [10-11]. Also, modification of some bioactive compounds have been implored to improve bioavailability, specificity and therapeutic effectiveness as well as other variety features that ~~which~~ include~~s~~ implementation of potential chemotherapeutic agents [12-14]. Propolis ~~which~~ is one of the natural product~~s~~ obtained from bees. It ~~has~~ve been acclaimed and reported as a medicinal product. It~~,~~ ~~which~~ is a complex resinous product having ~~many~~ compositions of phytochemicals which can change depending on collection site, botanical origin, climatice condition, trees around and extraction methods. Propolis has been used ethnomedically in ancient times as a remedy for variet~~yies~~ of disease~~s~~ and recently interest has been renewed in reinvestigating the drug potentials. It is also ~~has been~~ reported to have ~~antioxidant and~~ antitumor properties [15]. Flavonoids ~~which are the most common phytochemicals~~ that are commonly found in propolis ~~have been reported as phytochemicals~~ have~~ing~~ highest antioxidant, antitumor, cytotoxic and chemopreventive properties [16]. Some of the flavonoids that are ~~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]. Enzymes

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Poly (ADP-ribose) polymerases (PARPs) activate DNA repair mechanisms upon stress and cytotoxin-induced DNA damage and inhibition of PARP activity. This is a leading mechanism in cancer drug therapy[18]. PARP~~s~~-1 function~~s~~ as a DNA damage sensor and a signalling 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 in ~~to~~ both men and women.~~,~~ ~~i~~In terms of morbidity it is the third most common cancer while~~,~~ in terms of mortality it 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 these drugs ~~have been reported to~~ cause ~~have~~ some side effects on the patients. Therefore the search for natural alternative remains sacrosanct [20]. ~~with the help of~~ ~~m~~Molecular docking is a vital tool which ~~which have been~~ is used in computer aided drug design. ~~as a vital tool~~. It is one of the natural solutions towards this problem. ~~However~~ The present study has ~~this work will~~

focused 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.

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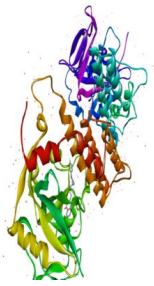
**Molecular Docking**

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.

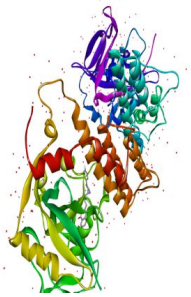
**Results and Discussion**

The result of the molecular docking of some flavonoids, terpenoids, drugs and the co-crystallized ligand on the protein of PARP are shown below below in the ~~t~~Table 1. Write about Fig 1 in the text.

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 |

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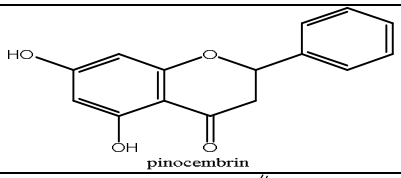
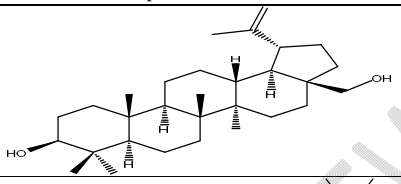
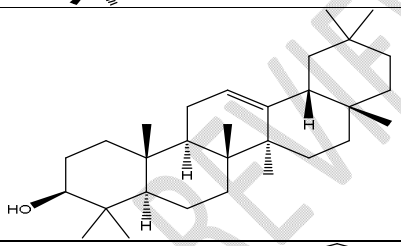
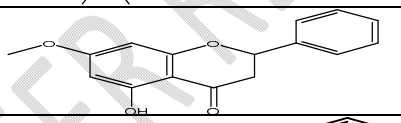
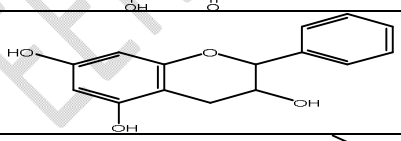
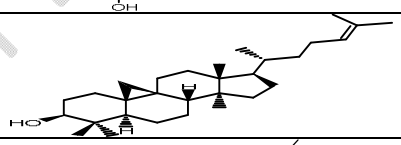
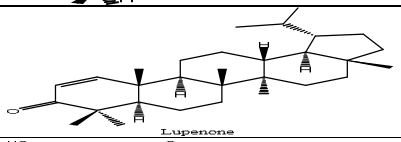
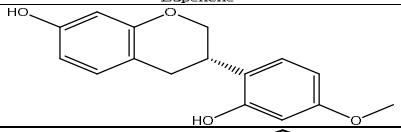
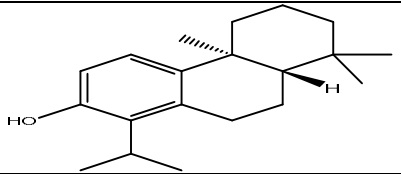
|---|---|---|---|
| Pinocembrin | 68071 |  | -8.9 |
| Betulin | 72326 |  | -9.7 |
| β-Amyrin | 73145 |  | -11.9 |
| Pinostrobin | 73201 |  | -8.8 |
| Pinobanksin | 73202 |  | -8.9 |
| Cycloartenol | 92110 |  | -11 |
| Lupenone | 92158 |  | -10.4 |
| Vestitol | 92503 |  | -8.8 |
| Totarol | 92783 |  | -10.2 |

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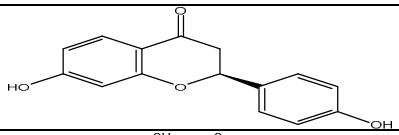
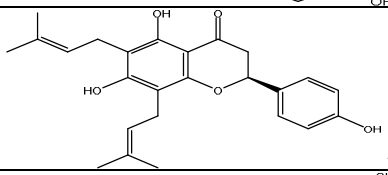
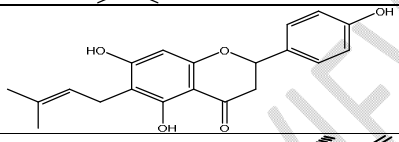
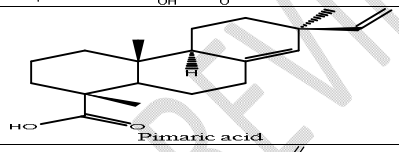
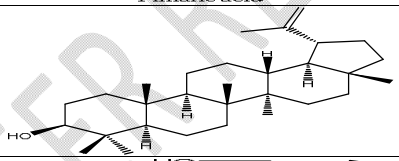
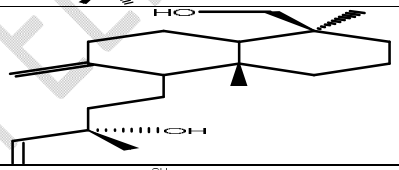
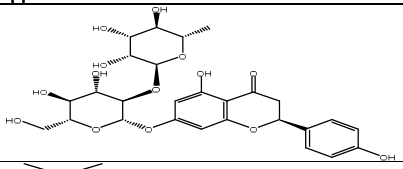
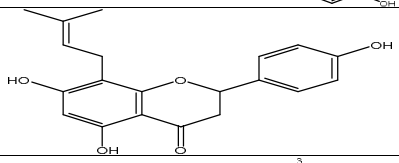
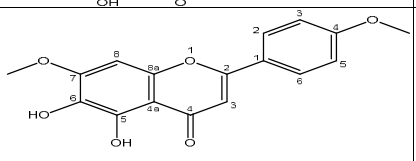
|---|---|---|---|
| Liquiritigenin | 114829 |  | -9.3 |
| Lonchocarpol A | 124035 |  | -10.7 |
| 6-prenylnaringenin | 155094 |  | -9.9 |
| Pimaric acid | 220338 |  | -8.9 |
| Lupeol | 259846 |  | -10 |
| Torulosol | 349315 |  | -7.7 |
| Naringin | 442428 |  | -11.7 |
| 8-prenylnaringenin | 480764 |  | -9.6 |
| Ladanein | 3084066 |  | -9.4 |

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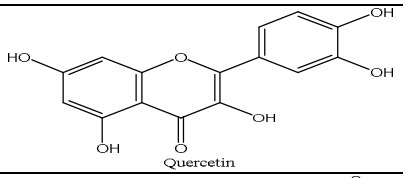
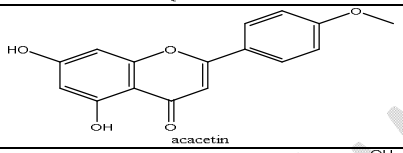
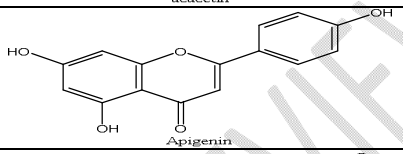
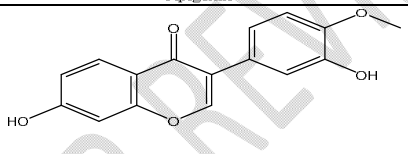
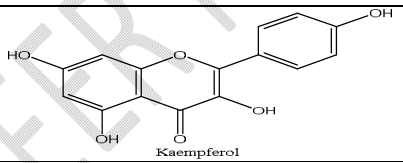
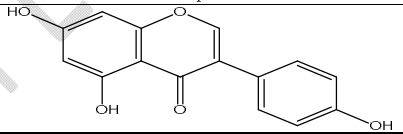
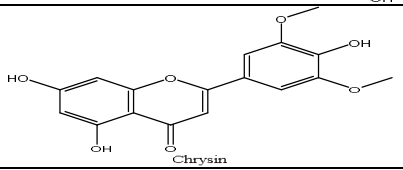
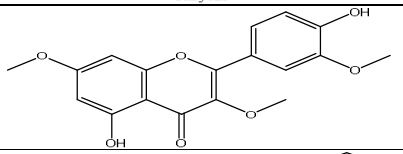
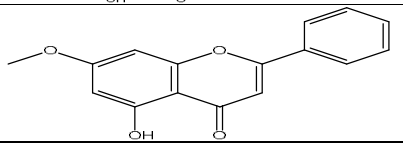
| Quercetin | 5280343 |  | -9.5 |
| Acacetin | 5280442 |  | -9.1 |
| Apigenin | 5280443 |  | -9 |
| Calycosin | 5280448 |  | -9.4 |
| Kaempferol | 5280863 |  | -9 |
| Genistein | 5280961 |  | -9.4 |
| Chrysin | 5281607 |  | -8.9 |
| Pachypodol | 5281677 |  | -8.7 |
| Tectrochrysin | 5281954 |  | -8.9 |

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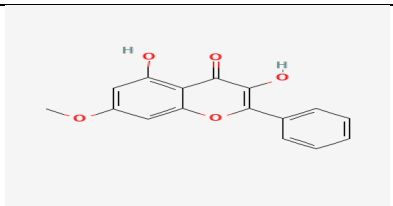
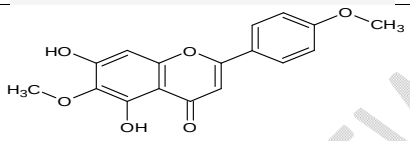
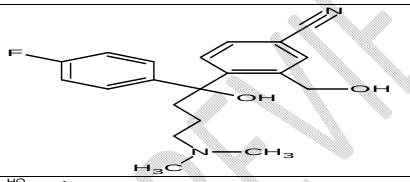
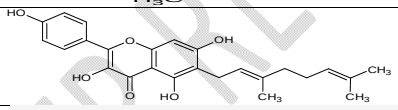
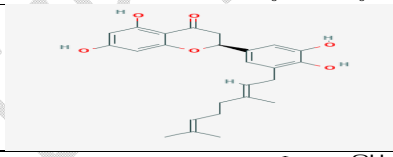
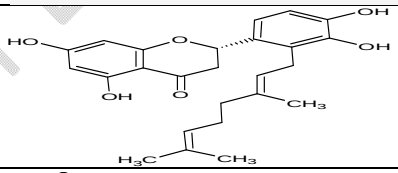
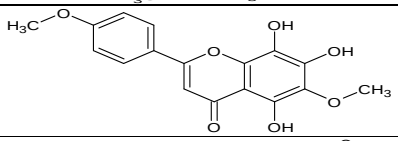
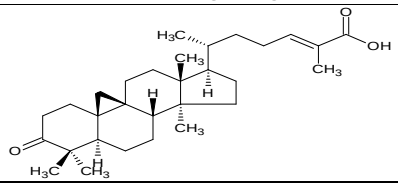
| Izalpinin | 5318691 |  | -8.7 |
| Pectolinarigenin | 5320438 |  | -9.1 |
| Citadiol | 7472055 |  | -8.3 |
| Macarangin | 10047854 |  | -10.3 |
| Isonymphaeol B | 10070991 |  | -11 |
| Nymphaeol B | 10387631 |  | -10.2 |
| Pilosin | 12085264 |  | -9.2 |
| Mangiferonic acid | 14034474 |  | -11.1 |

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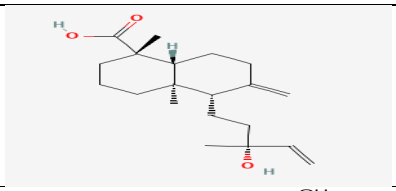
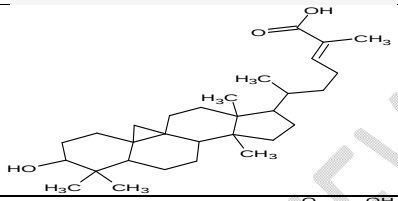
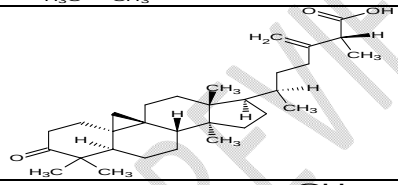
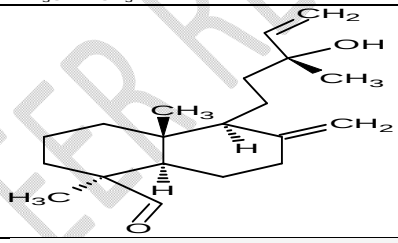
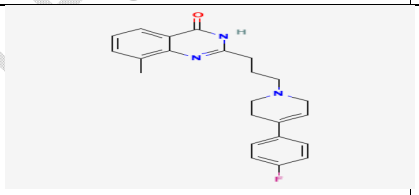
| Cupressic acid | 44584269 |  | -8.7 |
| Mangiferolic acid | 45270099 |  | -11 |
| Ambonic acid | 101286242 |  | -11.2 |
| Torulosal | 101306776 |  | -8.1 |
| Co-Ligand 2-{3-[4-(4-fluorophenyl)-3,6-dihydro-1(2h)-pyridinyl]propyl}-8-methyl-4(3h)-quinazolinone | 135460986 |  | -11.6 |

The binding energy of some compounds isolated from the African propolis ~~were~~ is shown in the table ~~1.1 which were~~ The compounds obtained were flavonoids and terpenoids but 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 co-crystallized ligand. The control drug Irinotecan had -11.2 binding activity while Doxorubicin had binding affinity of -9.1. The co-crystallized 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

**Comment [D4]:** Please check this. Mention the names of the compounds that were not analyzed.

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 co-crystallized ligand and drugs are shown in Fig 2

**Protein- Ligand interaction**

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

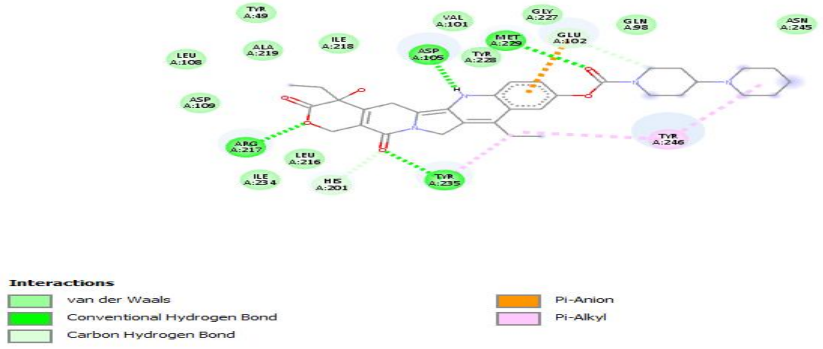



Figure 2.1: the interaction of the drug Irinotecan with the protein

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.

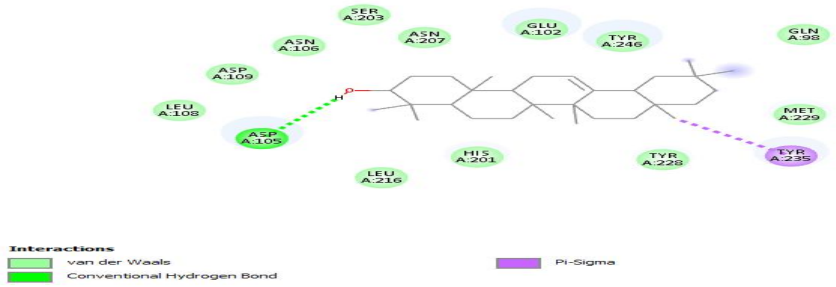



Figure 2.2: interaction of the protein with β-Amyrin

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.

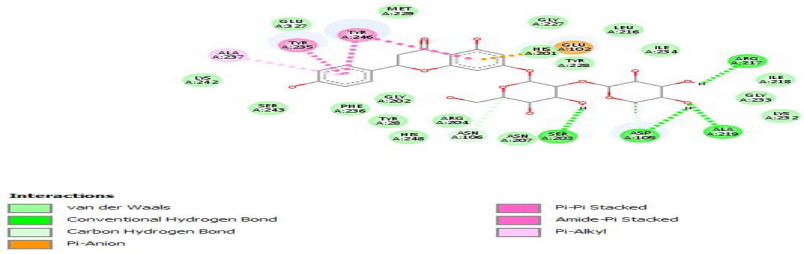



Fig 2.3: Interaction of the protein with Naringin

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

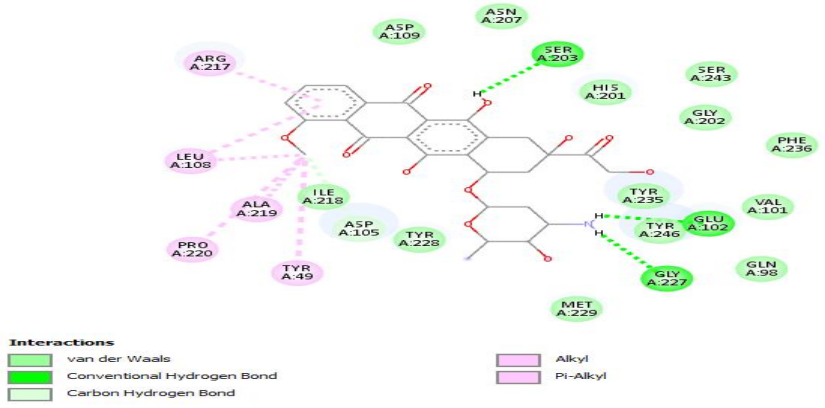



Fig 2.4: Interaction of the protein with Doxorubicin

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.

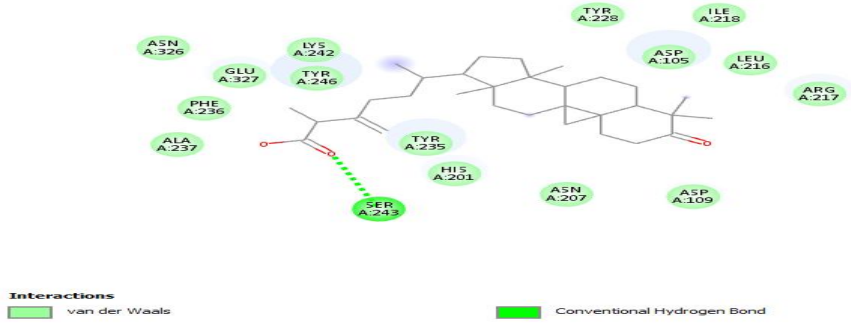



Fig 2.5: Protein interaction with Ambonic acid

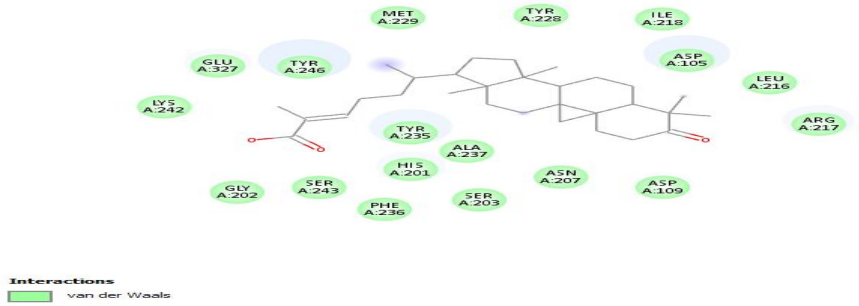



Fig 2.6: interaction with Mangiferonic acid

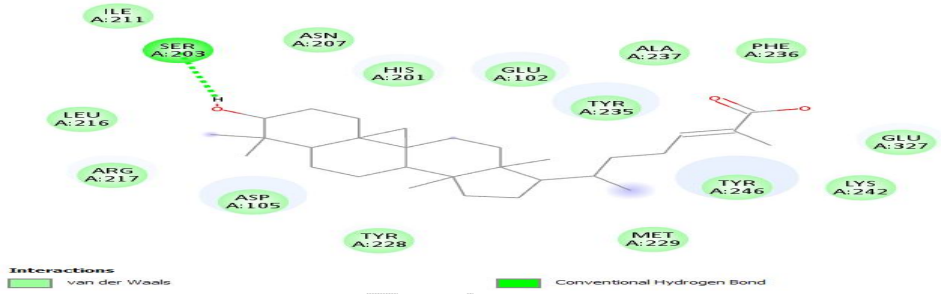



Fig 2.7: interaction with Mangniferolic acid

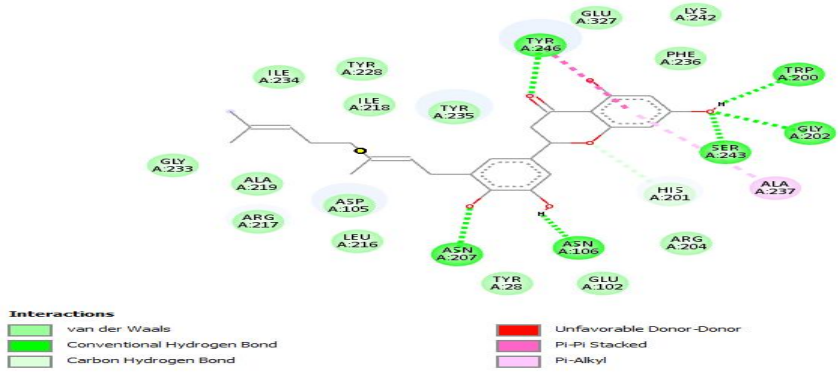



Fig 2.8: Interaction with Isonympeol A

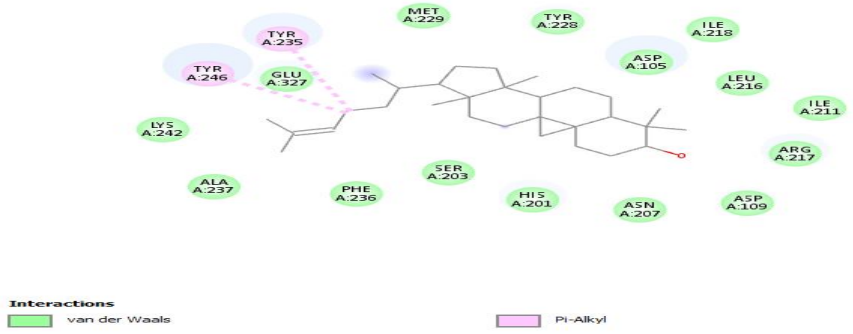



Fig 2.9: interaction with Cycloartenol

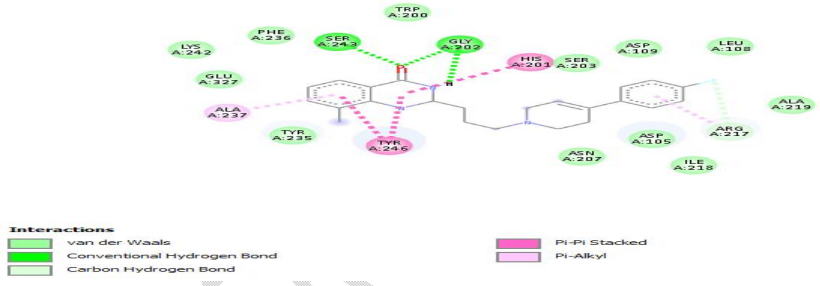



Fig 2.10: Co-crystallizied Ligand interaction with the protein

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.

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