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# GC-MS and Molecular Docking Studies of Crude Extract and Fraction of *Napoleonae Imperialis* as A Potent Antidiabetic Compound

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**ABSTRACT:**

**Background**: Peroxisome proliferator activated receptor gamma (PPAR-γ) agonists are beneficial in the treatment of diabetes by stimulating insulin sensitivity and antagonizing hepatic gluconeogenesis.

**Objective**: The aim of this study was to investigate the antidiabetic effect of phytocompounds synthesized from crude extract and fraction of *Napoleonae imperialis*, using GC-MS analysis and molecular docking studies.

**Methods**: Crude extract and fraction from *N. imperialis* were subjected to gas chromatography mass spectrometry (GC-MS) for identification of bioactive compounds in the plant and molecular docking of *Napoleonae imperialis* on human PPAR-γ protein was determined by Auto/Vina in Pymol 4.2 and compared with Glibenclamide, a known agonist of PPAR-γ.

**Results**: Our present study reports the phytochemical analysis of the crude extract and fraction from the leaves of *Napoleonae imperialis*. Sixty one (61) and eighty four (84) compounds were revealed through GC-MS analysis of the crude extract and fraction of *N. imperialis.* Molecular docking revealed that Dibutyl phthalate, 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and Guaiol bound favourably to the target receptor involved in glucose metabolism with a binding score of -7.3 kcal/mol and -7.5 kcal/mol against PPAR-γ.

**Conclusions**: *N. imperialis* methanol leaf extract and its bioactive compounds Dibutyl phthalate, 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and Guaiol had a significant antidiabetic activity against PPAR-γ. The molecular binding interaction of an in-silico data demonstrated that Guaiol has more specificity towards the PPAR-γ binding site and could be a potent antidiabetic compound.

**KEYWORDS**: Diabetes mellitus, *Napoleonae imperialis*, Guaiol, Molecular docking, Gas chromatography mass spectrometry, Peroxisome proliferator activated receptor gamma.

## INTRODUCTION

Diabetes mellitus, a medical condition associated with a defect in carbohydrate metabolism due to lack/deficiency of insulin secretion or a varying degree of insulin resistance could be managed successfully with dietary restrictions and herbal formulations.[1] According to World Health Organization, an estimated 422 million adults are living with diabetes mellitus.[2] In 2013, 381 million people have been revealed to be diabetic from the statistics of the International Diabetes Federation [3] and this figure is expected to increase by twofold in 2030. Several research studies have identified peroxisome proliferator activated receptor as key regulators of glucose and lipid metabolism [4,5], because they act as transcription factors activating protein synthesis in a wide variety of processes. Peroxisome proliferator-activated receptor gamma increases the activity of adipocyte glycerol kinase which consequently enhances glycerol integration into triglyceride. Glucose disposal in the peripheral tissue has been shown to be augmented by PPAR-γ which therefore increases the expression of the glucose transporter gene and glucose transporter 1 and [6,7]. Glibenclamide is a second generation sulfonylurea that reduces blood glucose by increasing insulin secretion from pancreatic beta cell [8], this helps to reduce the amount of sugar in the blood. Glibenclamide drugs produce several side effects, such as nausea, vomiting, constipation, diarrhea

and low blood sugar. There is a need to search for a new peroxisome proliferator-activated receptor gamma agonist with little or no side effect.

*Napoleonae imperialis* is a Nigerian folklore medicinal plant [9], that demonstrates antibacterial and wound healing properties in albino rats. [10] prepared an herbal ointment of the methanol solution of *Napoleonae imperialis* and examined its wound healing effect by the excision wound model on guinea pigs. The result of the experiment indicates that *Napoleonae imperialis* extract possess a better wound healing property as compared to the antibiotic used as control [11].

Recent research has shown that *Napoleonae imperialis* can be used to reduce glucose level in the blood of streptozotocin induced diabetic rats [12], but little has been done to show and authenticate the component in this leaf extract that functions as an efficacious antidiabetic compound. The objective of this study is to determine the antidiabetic agents present in the leaf extract of the plant by GC-MS and molecular docking analysis to find out the potent agent which can be employed in the management of diabetes.

## MATERIALS AND METHODS

### PLANT MATERIAL

Fresh leaves of the plant *Napoleonae imperialis* was obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and was identified by Prof. Garuba Omosun, a Taxonomist of the Plant Science and Biotechnology Department, Michael Okpara University of Agriculture, Umudike. The fresh leaves were washed and dried under shade at room temperature and were milled to fine powder using an electric blender (QLink, Model QBL, Taiwan) and stored in air tight containers.

### EXTRACTION

The powdered leaves of *Napoleonae imperialis*, 50 g, was soaked in 500 mL of methanol for 48 hours, after which the extract was filtered using a Whatman no. 1 filter paper and then the filtrate was air dried at a temperature of 40$^{o}$C and stored in the refrigerator for further use. The fraction from the crude extract was obtained by column fractionation.

### GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS

The gas chromatography mass spectrometry (GC-MS) analysis of the leaves of *Napoleonae imperialis* was performed using a GC-MS (Modal; Agilent technologies 7890A) equipped with a VF – 5ms fused silica capillary column of 30m length, 0.25 mm diameter and 0.25 mm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99 %) was used as carrier gas at a constant flow rate of 1 ml/min. Injection and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed from 80°C to hold for 2 mins at 10°C/min to 240°C to hold for 6 mins. 2 ml of water solution of the samples was manually inserted in the split less mode, with a split ratio of 1:40 and with a mass scan of 50 – 600 amu. The total running time of the GC-MS was 35 mins. The relative percentage of each extract constituent was expressed as a percentage with peak area normalization. Interpretation of the mass spectrum of the plant extracts was conducted using the database of the National Institute of Standard and Technology (NIST) library, having more than 62,000 spectral patterns. The spectra of the compounds were compared with the spectra of the National Institute of Standard and Technology (NIST) library database.

### MOLECULAR DOCKING

Molecular Docking is the process by which two molecules fit together in 3D space; it is a key tool in structural biology and computer aided drug design [13]. Auto Dock vina 4.0 [14] was used to carry out the molecular docking. The Auto Dock tool was used to calculate the ligand binding to PPAR gamma model using a grid spacing of 0.375 Å and the grid points in X, Y and Z axis were set at 60×60×60. The grid center coordinates were placed at X: 19.92, Y: 7.12, Z: 15.48. The grid boxes were placed at the binding site of the enzyme, which gives sufficient space for the ligand rotation and translation. The results obtained from Auto Dock were analyzed to study the binding energy and the interaction of the docked structure.

### PROTEIN PREPARATION

The 3D structure of PPAR gamma (PDB ID: 4EM9) was downloaded from the Protein Data Bank (PDB) (http://www.pdb.org/pdb/home/ home.do) before initiating the docking simulations. All non-protein molecules were removed from 4EM9; for any alternative atom locations, only the first location was retained. All the docking calculations were performed using AutoDock 4.0. PPAR gamma was modified by adding polar hydrogen and then kept rigid in the docking process, whereas all the torsional bonds of ligands were set free by the Ligand module in AutoDock Tools.

## GC-MS and Molecular Docking Studies of Crude Extract and Fraction of *Napoleonae Imperialis* as A Potent Antidiabetic Compound

### LIGAND PREPARATION

The 2D structure of the ligand isolated from the leaves of the plants was drawn using the ChemAxon software called MarvinSketch (https://www.chemaxon.com/). To prepare the ligand for docking, it was then converted to a 3-Dimensional structure with a force field of MMFF94.

### DOCKING CONFIRMATION USING MCULE

Mcule accelerates early-phase drug discovery by its integrated molecular modelling tools, computational capacity and high-quality target was uploaded in 3D with a binding site center X: 19.92, Y: 7.12, Z: 15.48. The simulation was run with a maximum hit of 1000.

### PCHEMBL CALCULATION

Pchembl calculation was done using Chembl (https://www.ebi.ac. uk/chembl/). The target fasta file was copied from pdb (http://www. rcsb.org/pdb/explore/explore.do? structure Id=4EM9) and uploaded at the Protein target BLAST search in Chembl. The target associated Bioactivities with Target ID of CHEMBL235 was downloaded. To calculate the Pchembl, the target associated Bioactivities was docked against PPAR gamma with a config.txt parameter. After docking, the results were harvested by 'egrep'.

### RESULTS

**Result of GC-MS analysis of methanol leaf extract of *Napoleonae imperialis***

**Table 1: GC-MS analysis of methanol leaf extract of *Napoleonae imperialis***

| Peak no | Compound | Molecular formula | Retention time | % composition | Molecular weight |
|---|---|---|---|---|---|
| 1 | 4-heptafluorobutyrloxyhexadecane | $C_{20}H_{33}F_7O_2$ | 5.214 | 0.09 | 433.50 |
| 2 | Cyclopropane, octyl | $C_{11}H_{22}$ | 5.367 | 0.13 | 154.29 |
| 3 | Benzene, 1-ethyl-3-methyl | $C_9H_{12}$ | 5.479 | 0.23 | 120.19 |
| 4 | Cycloheptane, methyl | $C_8H1_{16}$ | 5.930 | 0.39 | 112.21 |
| 5 | Decane | $C_{10}H_{22}$ | 6.367 | 1.44 | 142.28 |
| 6 | 5-Eicosene, (E) | $C_{20}H_{40}$ | 6.729 | 0.56 | 280.50 |
| 7 | E-15-Heptadecenal | $C_{17}H_{32}O$ | 6.967 | 0.67 | 252.40 |
| 8 | Dchloroacetic acid, pentadecyl ester | $C_{17}H_{33}ClO_2$ | 7.093 | 0.63 | 304.90 |
| 9 | 4-Propylcyclohexanone | $C_9H_{16}O$ | 7.192 | 0.49 | 140.22 |
| 10 | 1-Fluorononane | $C_9H_{17}F$ | 7.409 | 1.57 | 146.25 |
| 11 | Cyanoacetic acid, tetradecyl ester | $C_{17}H_{32}NO_2$ | 7.583 | 0.78 | 286.40 |
| 12 | Naphthalene, decahydro, trans | $C_{10}H_{18}$ | 7.815 | 2.05 | 138.25 |
| 13 | Decane, 1-fluoro | $C_{10}H_{21}F$ | 7.940 | 1.12 | 160.27 |
| 14 | 2-Trifluoroacetoxy tridecane | $C_{15}H_{27}F_3O_2$ | 8.106 | 2.30 | 296.37 |
| 15 | Oxalic acid, allyl undecyl ester | $C_{16}H_{28}O_4$ | 8.313 | 2.14 | 284.39 |
| 16 | 1-Nonadecene | $C_{19}H_{38}$ | 8.672 | 1.01 | 266.50 |
| 17 | Pentadec-7-ene,7-bromomethyl | $C_{16}H_{31}Br$ | 8.830 | 2.08 | 303.32 |
| 18 | Undecane | $C_{11}H_{24}$ | 9.168 | 0.57 | 156.31 |
| 19 | Naphthalene, decahydro-2-methyl | $C_{11}H_{20}$ | 9.408 | 0.22 | 152.28 |
| 20 | Dodecane | $C_{12}H_{26}$ | 12.090 | 0.13 | 170.33 |
| 21 | Heptadecane,8-methyl | $C_{18}H_{38}$ | 14.330 | 0.04 | 254.49 |
| 22 | 7-Tetradecene, (E)- | $C_{14}H_{28}$ | 17.566 | 0.04 | 196.37 |
| 23 | Tetradecane | $C_{14}H_{30}$ | 17.684 | 0.20 | 198.39 |
| 24 | Heptadecane | $C_{17}H_{36}$ | 20.101 | 0.10 | 240.50 |
| 25 | Hentriaacontane | $C_{31}H_{64}$ | 20.290 | 0.05 | 436.80 |
| 26 | Eicosane, 1-iodo | $C_{20}H_{41}I$ | 20.290 | 0.22 | 408.40 |
| 27 | Hexadecane | $C_{16}H_{32}$ | 22.754 | 0.59 | 226.44 |
| 28 | Epinephrine | $C_9H_{13}NO_3$ | 23.877 | 0.13 | 183.20 |
| 29 | Tetrapentacontane,1,54-dibromo | $C_{54}H_{108}Br_2$ | 24.243 | 0.08 | 917.20 |
| 30 | Hexadecane, 1-(ethenyloxy)- | $C_{18}H_{36}0$ | 24.400 | 0.15 | 268.49 |
| 31 | 4-methyl-z-tetradecen-1-ol acetate | $C_{17}H_{32}0$ | 24.943 | 0.38 | 268.40 |
| 32 | Dodecane,2-methyl | $C_{13}H_{28}$ | 25.095 | 0.40 | 184.36 |

| | | | | | |
|---|---|---|---|---|---|
| 33 | Heneicosane | $C_{21}H_{44}$ | 25.222 | 0.81 | 296.60 |
| 34 | Oxalic acid,3,5-difluorophenyl tetra ester | $C_{22}H_{32}F_2O_4$ | 25.427 | 0.26 | 398.50 |
| 35 | Octyl tetracosyl ether | $C_{32}H_{66}O$ | 25.542 | 0.41 | 466.90 |
| 36 | Oleic acid | $C_{18}H_{34}O_2$ | 25.895 | 0.71 | 282.50 |
| 37 | Octacosane | $C_{28}H_{58}$ | 26.212 | 1.71 | 394.80 |
| 38 | Octadecane | $C_{18}H_{34}O_2$ | 26.411 | 0.49 | 254.50 |
| 39 | 1-octadecanesulphonyl chloride | $C_{18}H_{37}ClO_2S$ | 26.497 | 0.47 | 353.00 |
| 40 | 2-methyl hexacosane | $C_{27}H_{56}$ | 26.639 | 1.30 | 380.70 |
| 41 | Nonadecane | $C_{19}H_{40}$ | 26.953 | 0.43 | 268.50 |
| 42 | 1-octadecene | $C_{18}H_{36}$ | 27.185 | 1.05 | 252.50 |
| 43 | Pentadecane | $C_{15}H_{32}$ | 27.320 | 1.36 | 212.41 |
| 44 | Cyclohexadecane | $C_{16}H_{32}$ | 27.477 | 1.16 | 224.42 |
| 45 | Heneicosane,11-pentyl | $C_{26}H_{54}$ | 29.151 | 0.07 | 366.70 |
| 46 | Eicosane | $C_{20}H_{42}$ | 29.391 | 0.09 | 282.50 |
| 47 | 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione | $C_{17}H_{24}O_3$ | 29.574 | 0.07 | 276.4 |
| 48 | Nonane,5-butyl | $C_{13}H_{28}$ | 29.887 | 0.08 | 184.36 |
| 49 | Dibutyl phthalate | $C_{16}H_{30}O_2$ | 30.003 | 0.05 | 278.34 |
| 50 | 1-Docosene | $C_{22}H_{44}$ | 30.224 | 0.33 | 308.60 |
| 51 | 13-Tetradecen-1-ol acetate | $C_{16}H_{30}O_2$ | 30.399 | 0.06 | 254.41 |
| 52 | Nonyl tetracosyl ether | $C_{33}H_{68}0$ | 31.100 | 0.37 | 480.89 |
| 53 | Triacontane,1-bromo | $C_{30}H_{61}Br$ | 31.348 | 0.33 | 501.7 |
| 54 | tert-Hexadecane thiol | $C_{48}H_{99}AUS_3$ | 31.640 | 0.59 | 969.50 |
| 55 | Pentafluoropropionic acid, hexadecyl ester | $C_{19}H_{33}F_5O_2$ | 31.770 | 0.87 | 388.50 |
| 56 | 13-Octadecanal (z)- | $C_{18}H_{34}O$ | 31.804 | 16.63 | 266.50 |
| 57 | Carbonic acid, but-2-yn-1-yl eicosyl ester | $C_{25}H_{46}O_3$ | 32.909 | 5.98 | 394.60 |
| 58 | Bis (tridecyl) phthalate | $C_{34}H_{58}O_4$ | 33.949 | 2.95 | 530.82 |
| 59 | 1-Hexacosene | $C_{26}H_{52}$ | 34.173 | 1.85 | 364.70 |
| 60 | 9-octadecenoic acid (z)-2,3-dihydroxyl propyl ester | $C_{21}H_{40}O_4$ | 34.683 | 17.62 | 356.54 |
| 61 | Squalene | $C_{30}H_{50}$ | 36.276 | 21.13 | 410.7 |

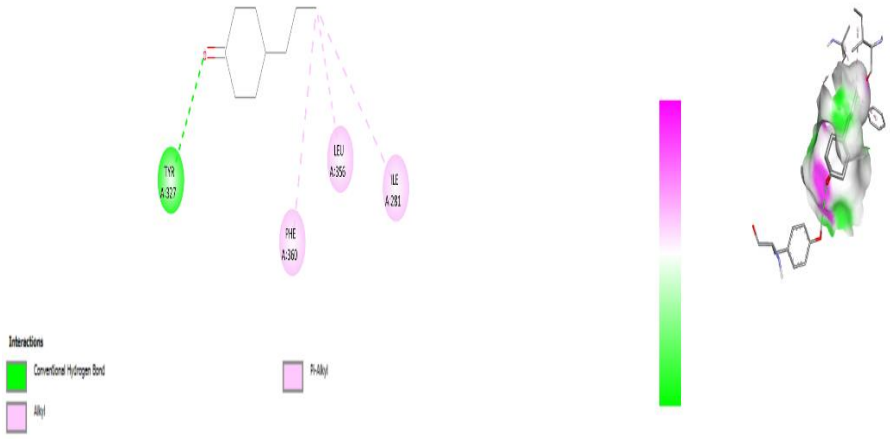
The GC-MS analysis of the methanol leaf extract of *Napoleonae imperialis* revealed the presence of sixty one (61) compounds which were confirmed based on the peak number, retention time, molecular weight and percentage composition.

**Result of molecular docking analysis of phytochemicals isolated from the methanol leaf extract of *Napoleonae imperialis* with peroxisome proliferator activated receptors (PPAR) gamma**

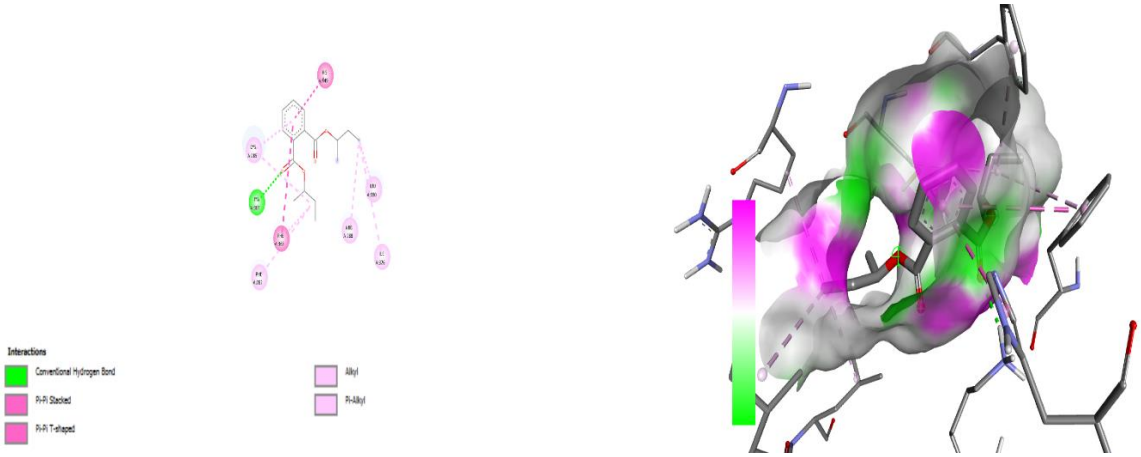
**Table 2: Binding analysis of phytochemicals isolated from the methanol leaf extract of *Napoleonae imperialis* with peroxisome proliferator activated receptors (PPAR) gamma**

| S/N | Compound | CID | Affinity (Kcal/mol) |
|---|---|---|---|
| \1 | 4-Propylcyclohexanone | 142482 | -6 |
| 2 | Di-sec-butyl phthalate | 249496 | -7.3 |
| 3 | Oleic acid | 445639 | -6.6 |
| 4 | 13-Tetradecen-1-ol acetate | 521718 | -5.9 |
| 5 | E-15-Heptadecenal | 5363097 | -6.3 |
| 6 | 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione | 545303 | -7.3 |
| 7 | Epinephrine | 5816 | -5.8 |
| 8 | Oxalic acid, allyl undecyl ester | 6420248 | -6 |

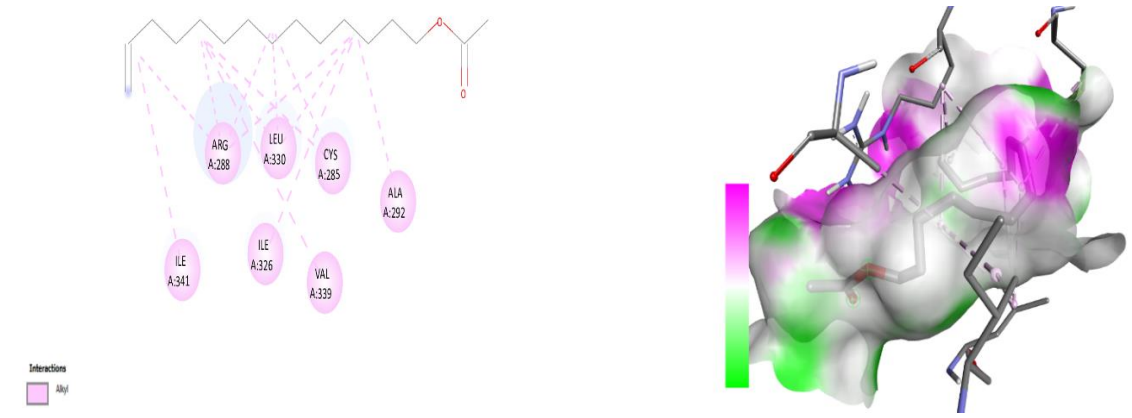
From the computational studies, the major bioactive constituents present in the methanol leaf extract of *Napoleonae imperialis* were docked against PPAR gamma, the receptor involved in insulin sensitivity pathway and their docking score calculated based on the gradient optimization algorithm using AutoDock Vina. Our findings revealed that the selected compounds were able to bind appreciably to the target protein. The docking scores (binding free energy, ΔG, kcal/mol) of the interaction are presented in Table 2 above. Analyses of the binding interactions was based on the ΔG of the best pose (the more negative the binding energy, the better the docking score). We observe that the compounds showed binding energy that ranged from -5.8 kcal/mol to -7.3 kcal/mol with PPAR gamma.



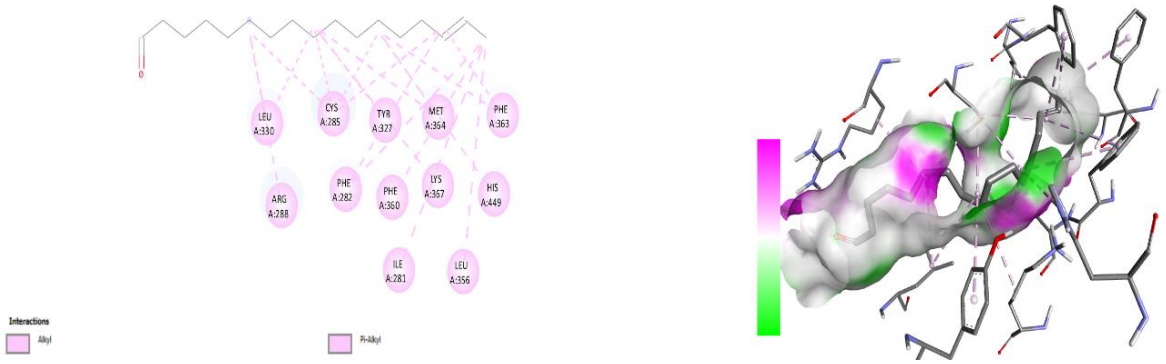

2D and 3D molecular interaction between 4-Propylcyclohexanone and PPAR gamma



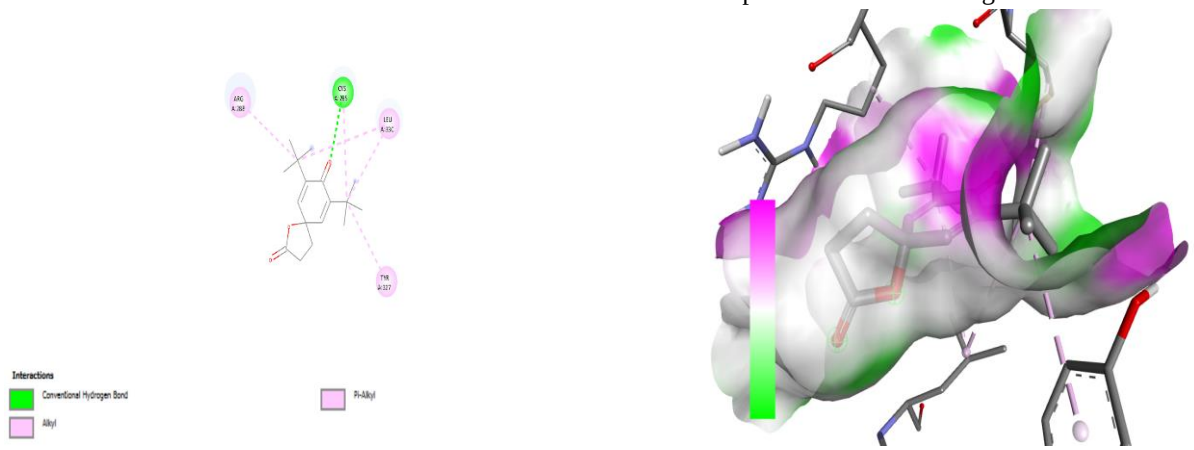

2D and 3D molecular interaction between of Di-sec-butyl phthalate with PPAR gamma



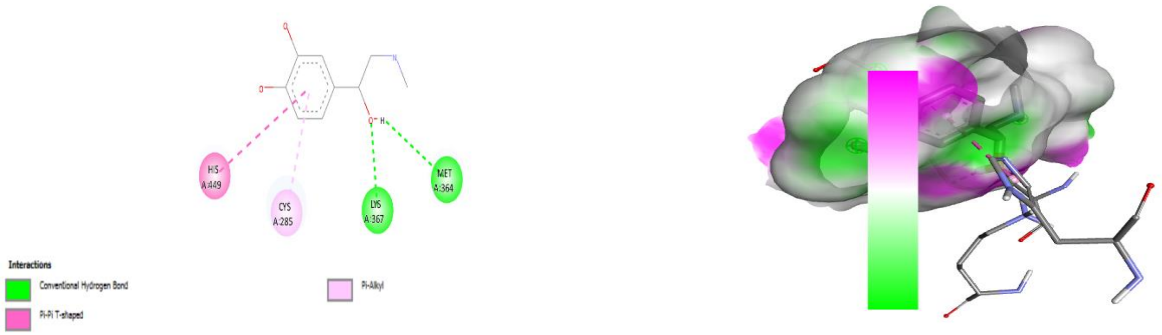

2D and 3D molecular interaction between 13-Tetradecen-1-0l aetate and PPAR gamma



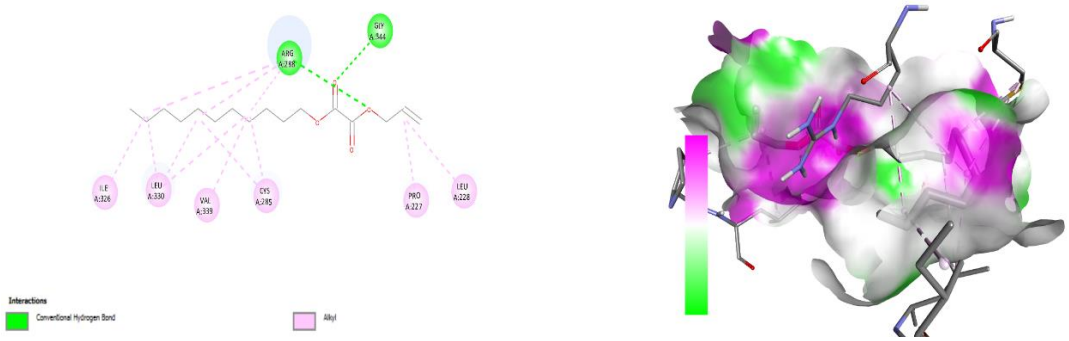

2D and 3D molecular interaction between E-15-Heptadecenal and PPAR gamma




2D and 3D molecular interaction between 7,9-ditert butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and PPAR gamma




2D and 3D molecular interaction between Epinephrine and PPAR gamma




2D and 3D molecular interaction between Oxalic acid, allyl undecyl ester and PPAR gamma

**Result of GC-MS analysis of the most potent fraction from methanol leaf extract of *Napoleonae imperialis***

**Table 3: GC-MS analysis of the most potent fraction from methanol leaf extract of *Napoleonae imperialis***

| Peak no | Compound | Molecular formula | Retention time | % composition | Molecular weight |
|---|---|---|---|---|---|
| 1 | 1-Propanesulfonyl chloride | $C_3H_7ClO_2S$ | 6.302 | 0.12 | 142.61 |
| 2 | Benzene,1,2,3-trimethyl | $C_9H_{12}$ | 6.367 | 0.29 | 120.19 |
| 3 | Decane | $C_{10}H_{22}$ | 6.496 | 0.33 | 142.28 |
| 4 | Benzene,1,4-dichloro | $C_6H_4Cl_2$ | 6.848 | 0.57 | 147.00 |
| 5 | 2-(chloromethyl)-5-ethyl-1,3,4 oxadiazole | $C_5H_7ClN_2O$ | 6.959 | 0.28 | 146.57 |
| 6 | trans-2-chlorovinylacetate | $C_4H_5ClO_2$ | 7.133 | 0.01 | 120.53 |
| 7 | Benzene,1-methyl-3-(1-methyl ethyl) | $C_{10}H_{14}$ | 7.202 | 0.23 | 134.22 |
| 8 | Oxalic acid, isobutyl nonyl ester | $C_{15}H_{28}O_4$ | 7.946 | 0.16 | 272.38 |
| 9 | gamma-Terpinene | $C_{10}H_{16}$ | 8.161 | 1.43 | 136.23 |
| 10 | Dodecane | $C_{12}H_{26}$ | 8.254 | 0.48 | 170.33 |
| 11 | Dodecane,2,6,11-trimethyl | $C_{15}H_{32}$ | 8.382 | 1.39 | 212.41 |
| 12 | Heptane,2,4,6-trimethyl | $C_{10}H_{22}$ | 8.537 | 1.37 | 142.28 |
| 13 | Carbonic acid nonyl vinyl ester | $C_{12}H_{22}O_3$ | 8.644 | 0.40 | 214.36 |
| 14 | Undecane,3,7-dimethyl | $C_{13}H_{28}$ | 8.699 | 0.64 | 184.36 |
| 15 | Hexadecane | $C_{16}H_{32}$ | 8.785 | 0.42 | 226.44 |
| 16 | Dodecane | $C_{12}H_{26}$ | 8.958 | 3.22 | 170.33 |
| 17 | Tridecane, 6-methyl | $C_{14}H_{30}$ | 9.120 | 0.81 | 198.39 |
| 18 | Hexane, 3,3-dimethyl | $C_8H_{18}$ | 9.176 | 1.37 | 114.23 |
| 19 | Oxalic acid, 2-ethylhexyl isohexyl ester | $C_{16}H_{30}O_4$ | 9.272 | 1.45 | 286.41 |
| 20 | Carbonic acid, prop-1-ene-2-yl tridecyl ester | $C_{17}H_{32}O_3$ | 9.340 | 1.45 | 284.40 |
| 21 | Linalool | $C_{10}H_{18}O$ | 9.438 | 1.92 | 154.25 |
| 22 | Heptane, 2,4-dimethyl | $C_9H_{20}$ | 9.638 | 0.62 | 128.25 |
| 23 | Octane, 2,3,3-trimethyl | $C_{11}H_{24}$ | 9.691 | 0.78 | 156.31 |
| 24 | Octane,2,6-dimethyl | $C_{10}H_{22}$ | 9.532 | 1.34 | 142.28 |
| 25 | Nonane,3-methyl | $C_{10}H_{22}$ | 9.808 | 1.06 | 142.28 |
| 26 | Undecane | $C_{11}H_{24}$ | 9.917 | 0.32 | 156.31 |
| 27 | 2,6-Dimethyl decane | $C_{12}H_{26}$ | 10.027 | 0.72 | 170.33 |
| 28 | Tetratetracontane | $C_{44}H_{90}$ | 10.101 | 0.66 | 619.20 |
| 29 | Octane,3,4,6,6-tetramethyl | $C_{12}H_{26}$ | 10.156 | 0.81 | 170.33 |
| 30 | Hydroxylamine, o-decyl | $C_{10}H_{23}NO$ | 10.851 | 0.25 | 173.30 |
| 31 | Terpinen-4-ol | $C_{10}H_{18}O$ | 11.652 | 0.50 | 154.25 |
| 32 | Azulene | $C_{10}H_8$ | 11.772 | 0.30 | 128.17 |
| 33 | 5-Dodecene | $C_{12}H_{24}$ | 12.027 | 0.40 | 168.32 |
| 34 | Benzocycloheptatriene | $C_{11}H_{10}$ | 14.941 | 0.24 | 142.20 |
| 35 | Tridecane | $C_{13}H_{28}$ | 15.110 | 0.80 | 184.36 |
| 36 | Sulfurous acid, butyl dodecyl ester | $C_{16}H_{34}O_3S$ | 16.849 | 0.12 | 306.50 |
| 37 | .alfa – copaene | $C_{15}H_{24}$ | 17.182 | 0.87 | 204.35 |

| | | | | | |
|---|---|---|---|---|---|
| 38 | 1,3-Pentadiene, (z)- | $C_5H_8$ | 17.639 | 2.17 | 68.187 |
| 39 | Hexadecane | $C_{16}H_{34}$ | 17.831 | 0.76 | 226.44 |
| 40 | 1H-cycloprop(e) azulene, 1a,2,3,4,4a,5 6,7b-octahydro-1,1,4,7-tetrane | $C_{15}H_{24}$ | 18.081 | 0.20 | 204.35 |
| 41 | Santolina triene | $C_{10}H_{16}$ | 18.735 | 0.43 | 136.23 |
| 42 | Humulene | $C_{15}H_{24}$ | 19.248 | 1.41 | 204.36 |
| 43 | E-beta-famesene | $C_{15}H_{24}$ | 19.354 | 2.75 | 204.35 |
| 44 | Aromandendrene | $C_{15}H_{24}$ | 19.474 | 0.56 | 204.35 |
| 45 | 1-(3,3-Dimethyl butyn-1-yl)-2,2-dimethyl cyclopropene | $C_{11}H_{16}$ | 19.868 | 0.45 | 148.24 |
| 46 | beta,- Copaene | $C_{15}H_{24}$ | 19.973 | 2.63 | 204.35 |
| 47 | alpha,- Muurolene | $C_{15}H_{24}$ | 20.480 | 0.26 | 204.35 |
| 48 | Bicyclo (4.1.0) heptane-3-cyclopropyl -7- hydroxy methyl (cis) | $C_{11}H_8O$ | 20.585 | 0.24 | 166.25 |
| 49 | beta,- Bisabolene | $C_{15}H_{24}$ | 20.696 | 6.94 | 204.35 |
| 50 | 2,4-Di-tert-butylphenol | $C_{14}H_{22}O$ | 20.981 | 2.22 | 206.32 |
| 51 | -beta,- Ocimene | $C_{10}H_{16}$ | 21.530 | 0.26 | 136.23 |
| 52 | Cyclohexanemethanol,4-ethenyl, alpha 4-trimethyl-3-(1-methyl ethenyl)- [1R-(1-alpha, 3-alpha, 4-beta)] | $C_{15}H_{26}O$ | 21.749 | 0.25 | 223.37 |
| 53 | Alloaromadendrene | $C_{15}H_{24}$ | 21.875 | 0.70 | 204.35 |
| 54 | 1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl (Nerolidol) | $C_{15}H_{26}O$ | 22.075 | 2.25 | 222.37 |
| 55 | Caryophyllene oxide | $C_{15}H_{24}O$ | 22.533 | 0.70 | 220.35 |
| 56 | 4-Heptafluorobutyryloxy hexadecane | $C_{20}H_{33}F_7O_2$ | 22.690 | 1.65 | 438.50 |
| 57 | 10-Methyl nonadecane | $C_{20}H_{42}$ | 22.858 | 0.20 | 282.55 |
| 58 | Guaiol | $C_{15}H_{26}O$ | 22.926 | 0.71 | 222.37 |
| 59 | 3-cyclohexen-1-carboxaldehyde 3,4-dimethyl | $C_9H_{14}O$ | 23.181 | 0.22 | 138.21 |
| 60 | Apiol | $C_{12}H_{14}O_4$ | 23.597 | 0.21 | 222.24 |
| 61 | 1H-3a,7-Methano azulene, octahydro-1,4,9,9-tetramethyl | $C_{15}H_{26}$ | 23.691 | 0.37 | 206.37 |
| 62 | tau.- Mourolol | $C_{15}H_{26}O$ | 23.987 | 0.51 | 222.37 |
| 63 | Preg-4-en-3-one, 17. alpha. hydroxy-17. beta .-cyano | $C_{20}H_{27}NO_2$ | 25.064 | 0.67 | 313.44 |
| 64 | 2-Heptanone-1-ethoxy | $C_9H_{18}O_2$ | 25.261 | 0.42 | 158.24 |
| 65 | 1-Octadecene | $C_{18}H_{36}$ | 27.256 | 1.43 | 252.50 |
| 66 | Undec-10-ynoic acid, tretradecyl ester | $C_{25}H_{46}O_2$ | 27.788 | 0.20 | 378.60 |
| 67 | 13-Tetradece-11-yn-1-ol | $C_{14}H_{24}O$ | 28.364 | 0.16 | 208.34 |
| 68 | 2-[2-Thienyl] Cinchoninic acid ethyl ester | $C_{16}H_{13}N0_2S$ | 29.204 | 0.15 | 283.30 |
| 69 | Dimethyl bicyclo [2.2.1] hepta-2,5-2,5-diene-2,3-dicarboxylate | $C_{11}H_{12}O_4$ | 29.766 | 0.69 | 208.21 |
| 70 | Di-sec-butyl phthalate | $C_{16}H_{22}O_4$ | 30.016 | 0.59 | 278.34 |
| 71 | Hexadecanoic acid, ethyl ester | $C_{18}H_{36}O_2$ | 30.281 | 2.46 | 284.50 |
| 72 | 5- Eicosene, (E)- | $C_{20}H_{40}$ | 30.359 | 0.09 | 280.50 |
| 73 | Acetoxyacetic acid, tridec-2-ynyl ester | $C_{17}H_{28}O_4$ | 30.560 | 0.11 | 296.40 |
| 74 | Cyclododecane, ethyl | $C_{14}H_{28}$ | 31.080 | 0.13 | 196.37 |
| 75 | Dodecane, 1-bromo | $C_{12}H_{25}Br$ | 31.148 | 0.14 | 249.23 |
| 76 | 1- Methyl cyclo heptanol | $C_8H_{16}O$ | 31.305 | 0.16 | 128.21 |
| 77 | Linoleic acid ethyl ester | $C_{20}H_{36}O_2$ | 31.629 | 0.95 | 308.50 |
| 78 | 9- Octadecenoic, ethyl ester | $C_{20}H_{38}O_2$ | 31.662 | 0.88 | 310.51 |
| 79 | 1-Docosene | $C_{22}H_{44}$ | 31.813 | 0.90 | 308.58 |
| 80 | 2- Methyl- z, z-3,13-octadecadienol | $C_{19}H_{36}O$ | 32.482 | 0.12 | 280.49 |

| | | | | | |
|---|---|---|---|---|---|
| 81 | 2- Heptene, (E)- | $C_7H_{14}$ | 32.611 | 0.27 | 98.19 |
| 82 | Heptadecyl trifluoroacetate | $C_{19}H_{35}F_3O_2$ | 32.967 | 0.38 | 352.48 |
| 83 | Bis (2-ethyl hexyl) phthalate | $C_{24}H_{38}O_4$ | 34.003 | 0.77 | 390.56 |
| 84 | Palmitoleic acid | $C_{16}H_{30}O_2$ | 34.140 | 0.24 | 254.41 |

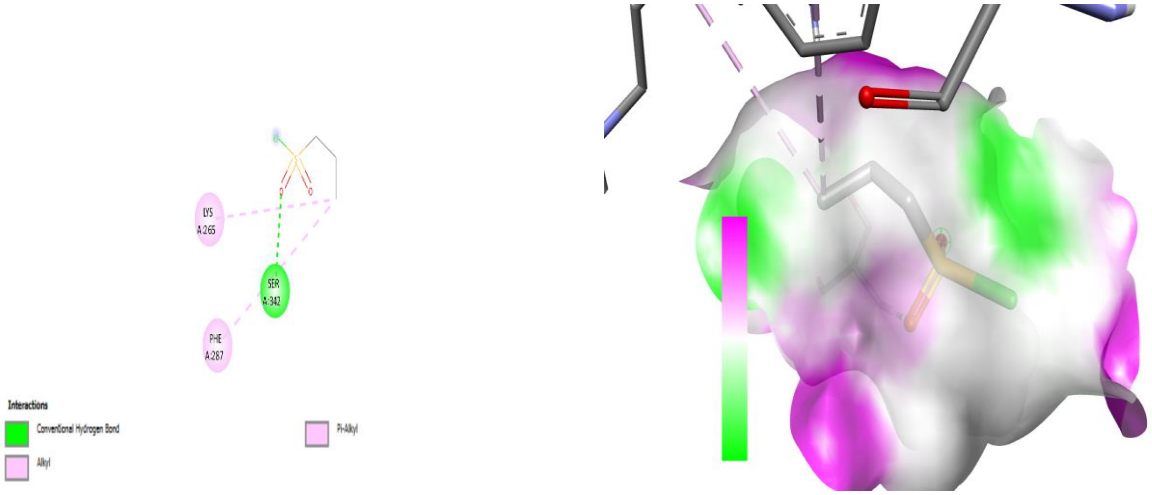
The GC-MS analysis of the most potent fraction from the methanol leaf extract of *Napoleonae imperialis* revealed the presence of eighty four (84) compounds which was confirmed based on the peak number, retention time, molecular weight and percentage composition.

**Result of molecular docking analysis of the most potent fraction of the methanol leaf extract of *Napoleonae imperialis* with PPAR gamma.**

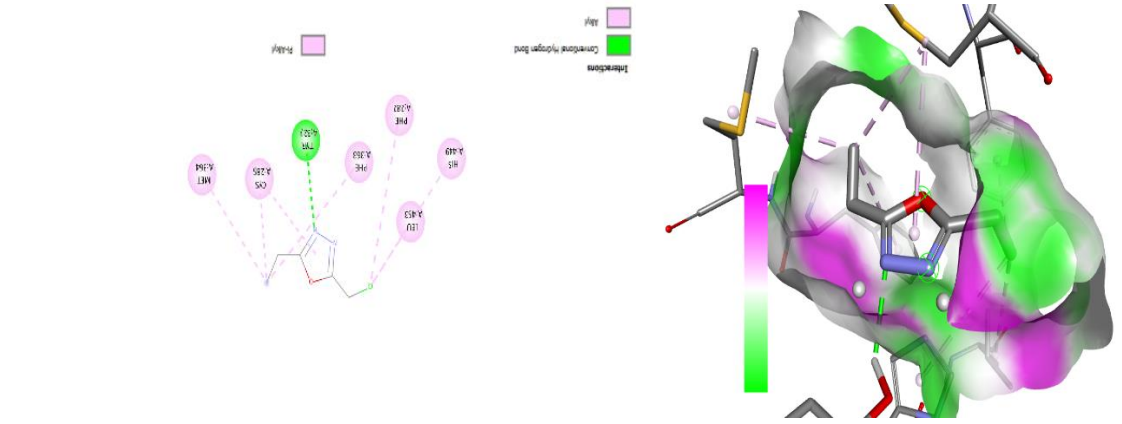
T**able.4: Binding analysis of phytochemicals isolated from the most potent fraction of the methanol leaf extract of *Napoleonae imperialis* with PPAR gamma.**

| S/N | Compound | CID | Affinity (Kcal/mol) |
|---|---|---|---|
| \1 | 1-Propanesulfonyl chloride | 66279 | -3.9 |
| 2 | 2-(chloromethyl-5-ethyl-1,3.4) oxadiazole | 16227319 | -5.2 |
| 3 | 2- Heptanone-1-ethoxy | 39913 | -5 |
| 4 | 3- Cyclohexen-1-carboxaldehyde,3,4-dimethyl | 537551 | -5.2 |
| 5 | 13-Tetradece-11-yn-1-ol | 543337 | -5.7 |
| 6 | 2,4- Ditert butyl phenol | 7311 | -7 |
| 7 | Apiol | 10659 | -6.6 |
| 8 | Carbonic acid | 91692935 | -6.4 |
| 9 | Carbonic acid nonyl vinyl ester | 91691497 | -5.7 |
| 10 | Caryophyllene oxide | 1742210 | -6.5 |
| 11 | Guaiol | 227829 | -7.5 |
| 12 | Hydroxylamine, o-decyl | 34704 | -5.5 |
| 13 | Linalool | 6549 | -5.7 |
| 14 | Oxalic acid, 2-ethylhexyl isohexyl ester | 6420724 | -6.8 |
| 15 | Oxalic acid, isobutyl nonyl ester | 6420705 | -6.4 |
| 16 | Terpinen-4-ol | 11230 | -5.6 |
| 17 | trans-2-chlorovinyl acetate | 14876280 | -3.9 |

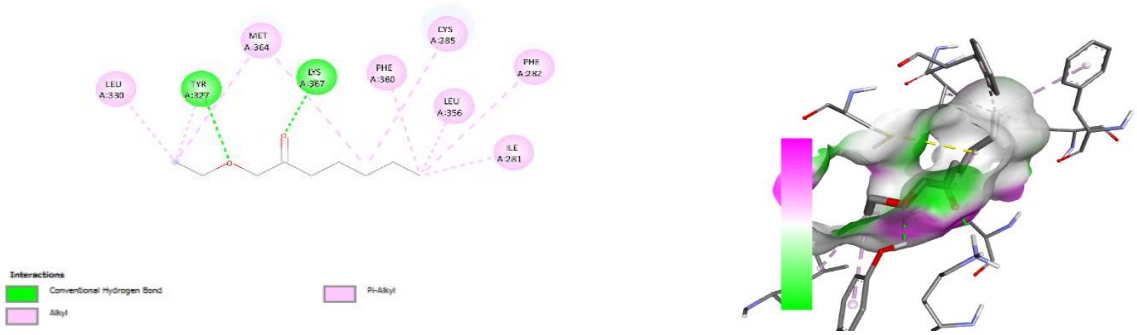
From the computational studies, the major bioactive constituents present in the methanol leaf extract of *Napoleonae imperialis* was docked against PPAR gamma, the receptor involved in insulin sensitivity pathway and their docking score calculated based on the gradient optimization algorithm using AutoDock Vina. Our findings revealed that the selected compounds were able to bind appreciably to the target protein. The docking scores (binding free energy, ΔG, kcal/mol) of the interaction are presented in Table 4 above. Analyses of the binding interactions was based on the ΔG of the best pose (the more negative the binding energy, the better the docking score). We observe that the compound showed binding energy that ranged from -3.9 kcal/mol to -7.5 kcal/mol with PPAR gamma.



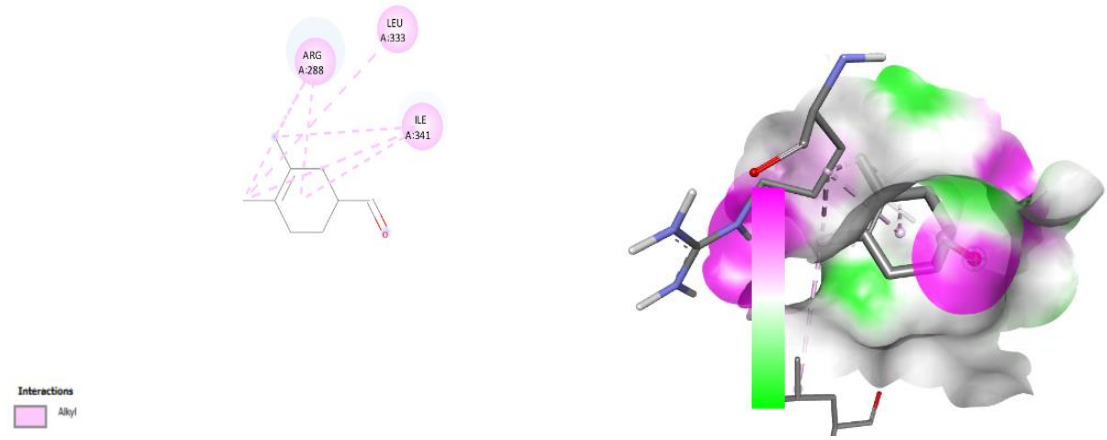

2D and 3D molecular interaction between 1-Propanesulfonyl chloride and PPAR gamma



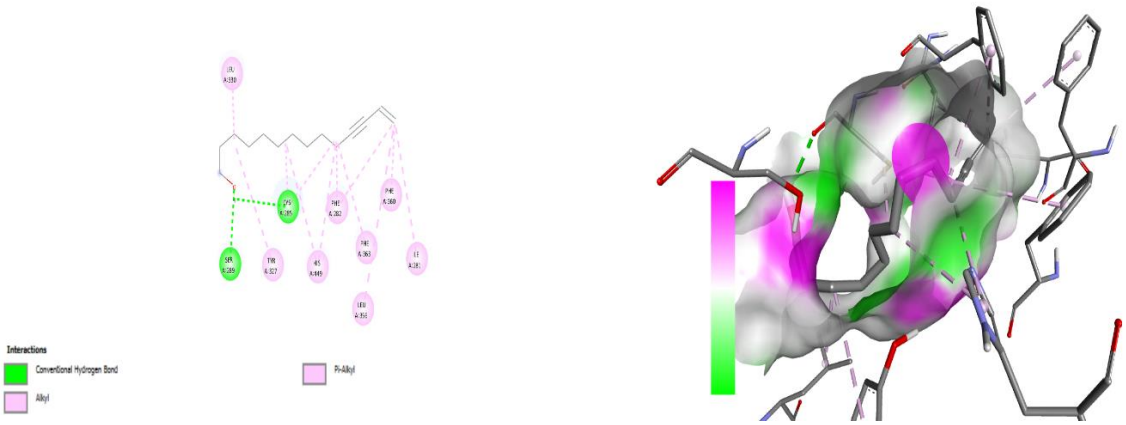
2D and 3D molecular interaction between 2-(chloromethyl-5-ethyl-1,3.4) oxadiazole and PPAR gamma



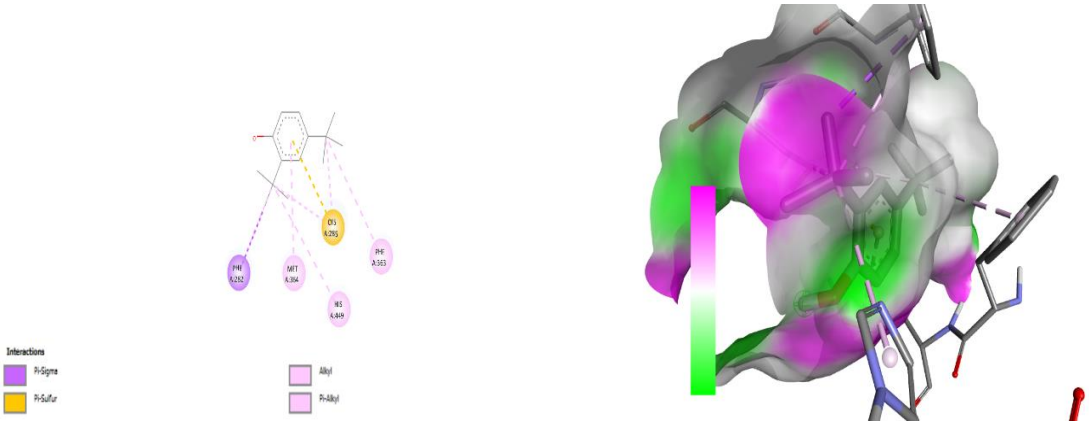

2D and 3D molecular interaction between 2- Heptanone-1-ethoxy and PPAR gamma



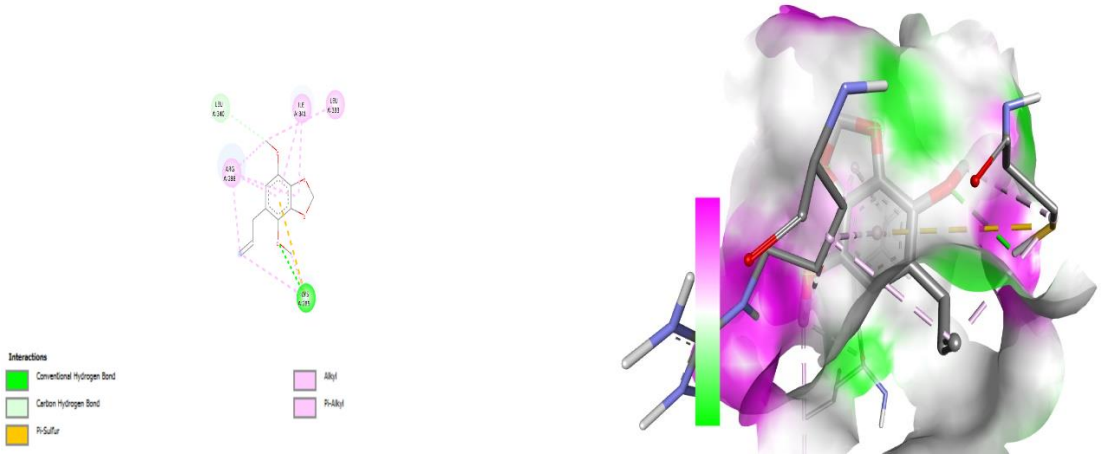

2D and 3D molecular interaction between 3- Cyclohexen-1-carboxaldehyde,3,4-dimethyl and PPAR gamma



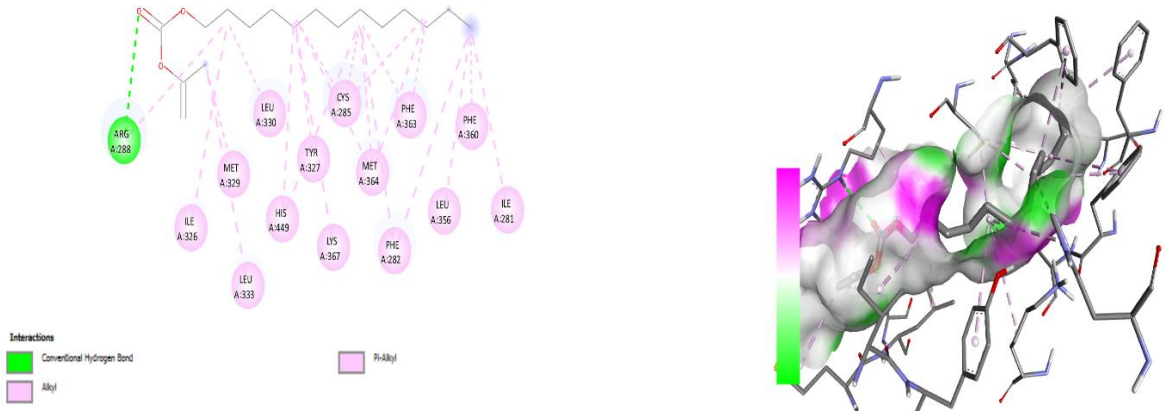

2D and 3D molecular interaction between 13-Tetradece-11-yn-1-ol and PPAR gamma



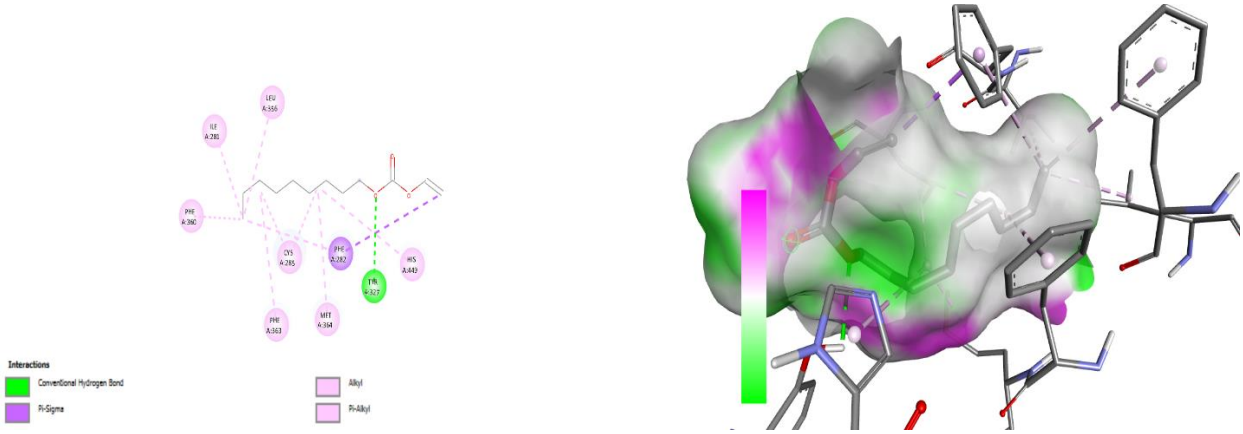

2D and 3D molecular interaction between 2,4- Ditert butyl phenol and PPAR gamma



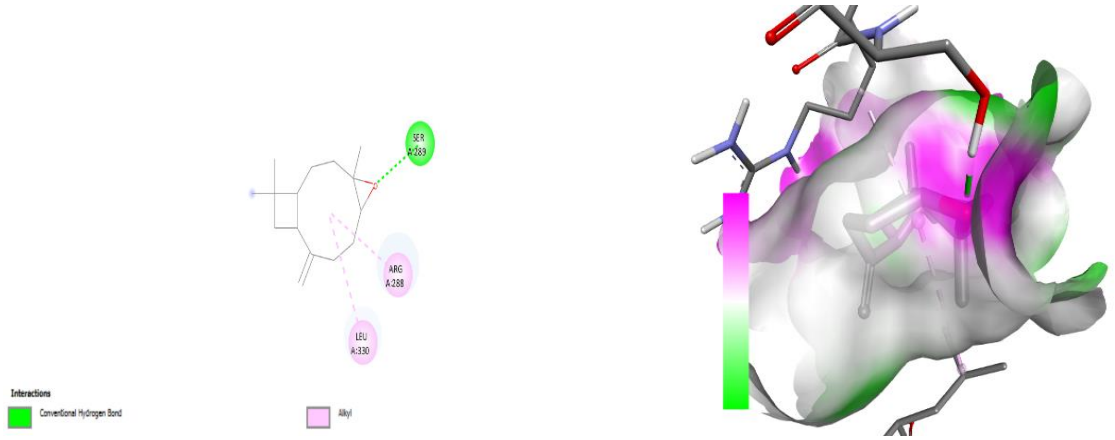

2D and 3D molecular interaction between Apiol and PPAR gamma



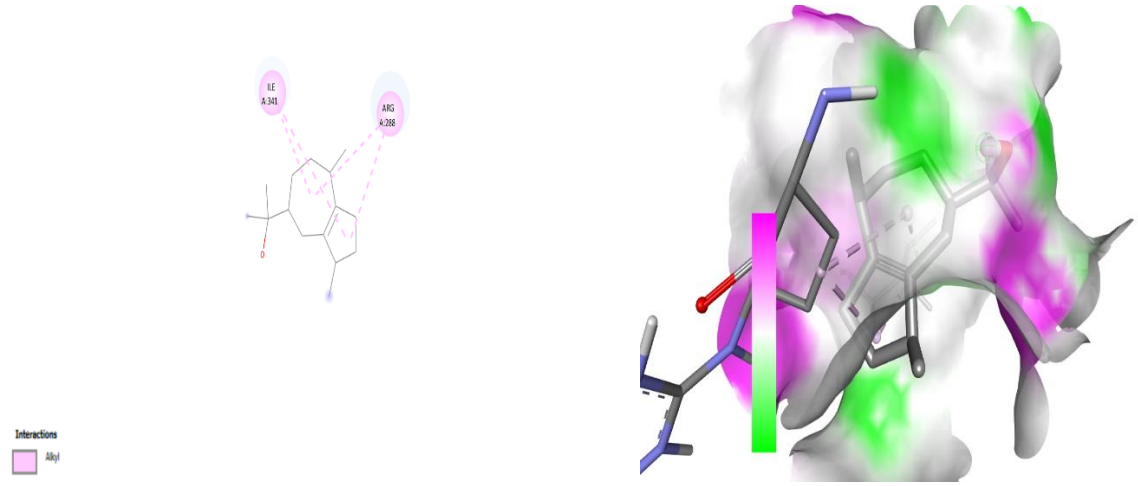

2D and 3D molecular interaction between Carbonic acid and PPAR gamma



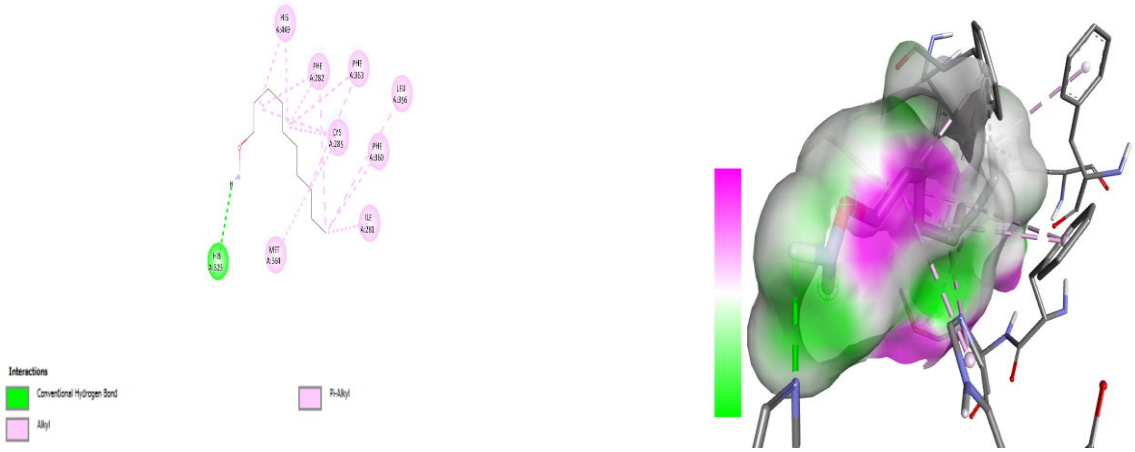

2D and 3D molecular interaction between Carbonic acid nonyl vinyl ester and PPAR gamma



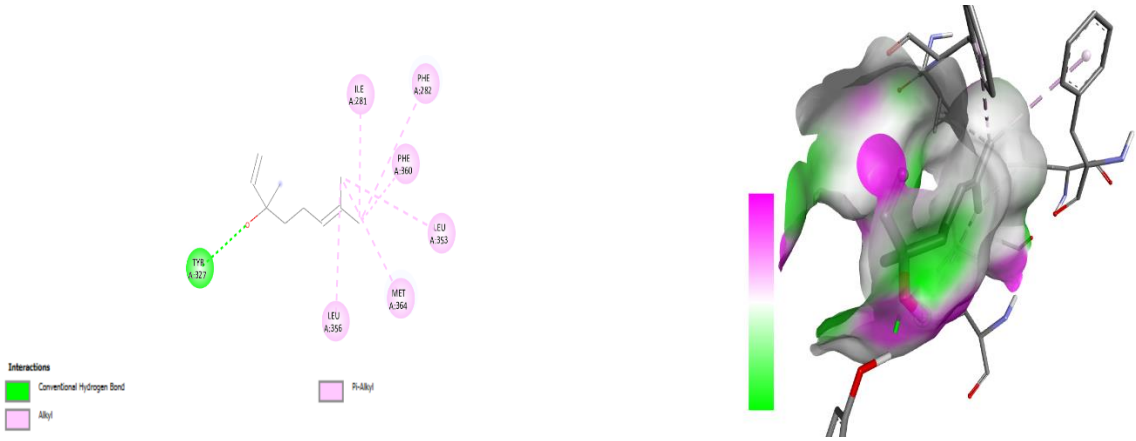

2D and 3D molecular interaction between Caryophyllene oxide and PPAR gamma



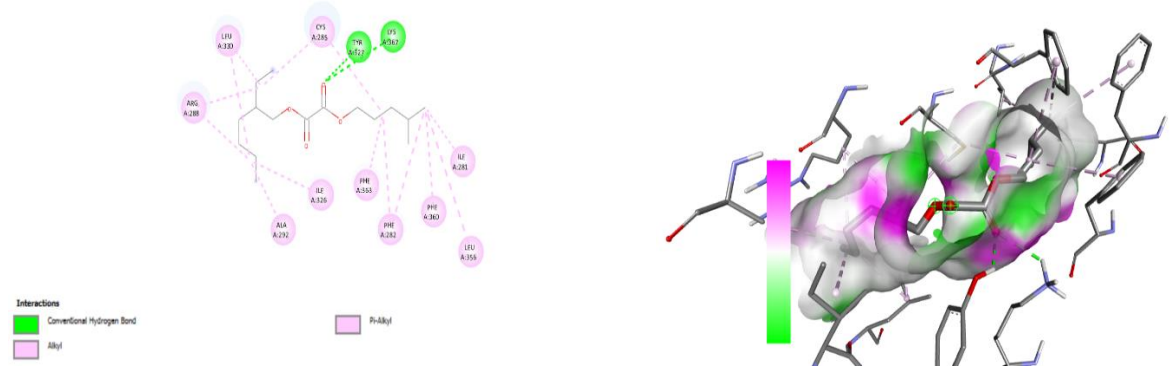

2D and 3D molecular interaction between Guaiol and PPAR gamma



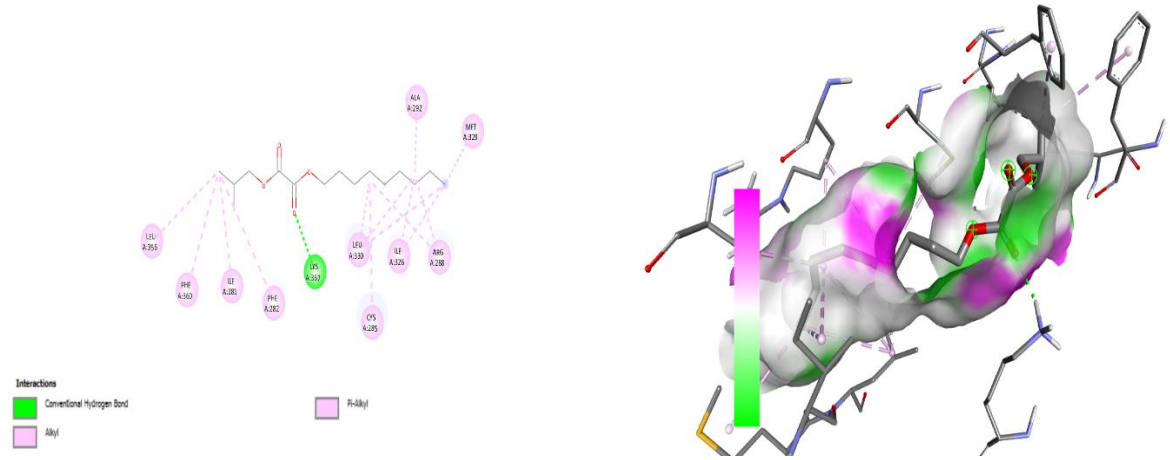

2D and 3D molecular interaction between Hydroxylamine, o-decyl and PPAR gamma



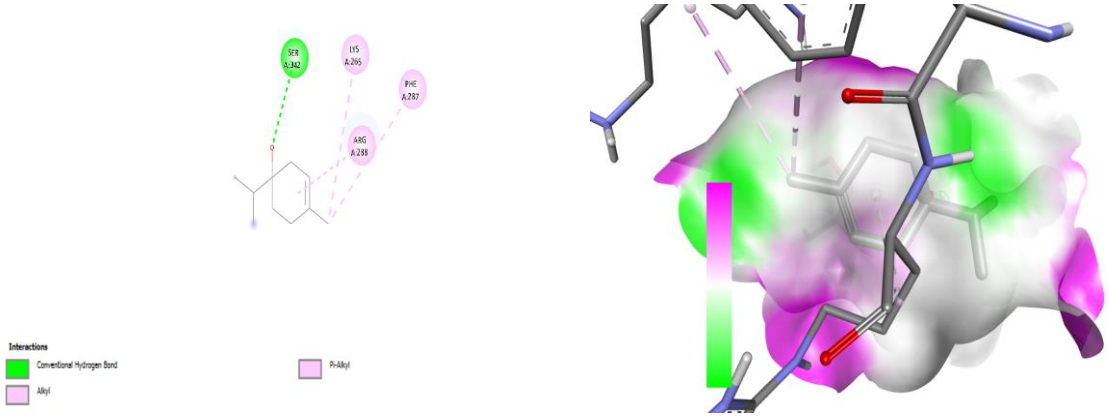

2D and 3D molecular interaction between Linalool and PPAR gamma



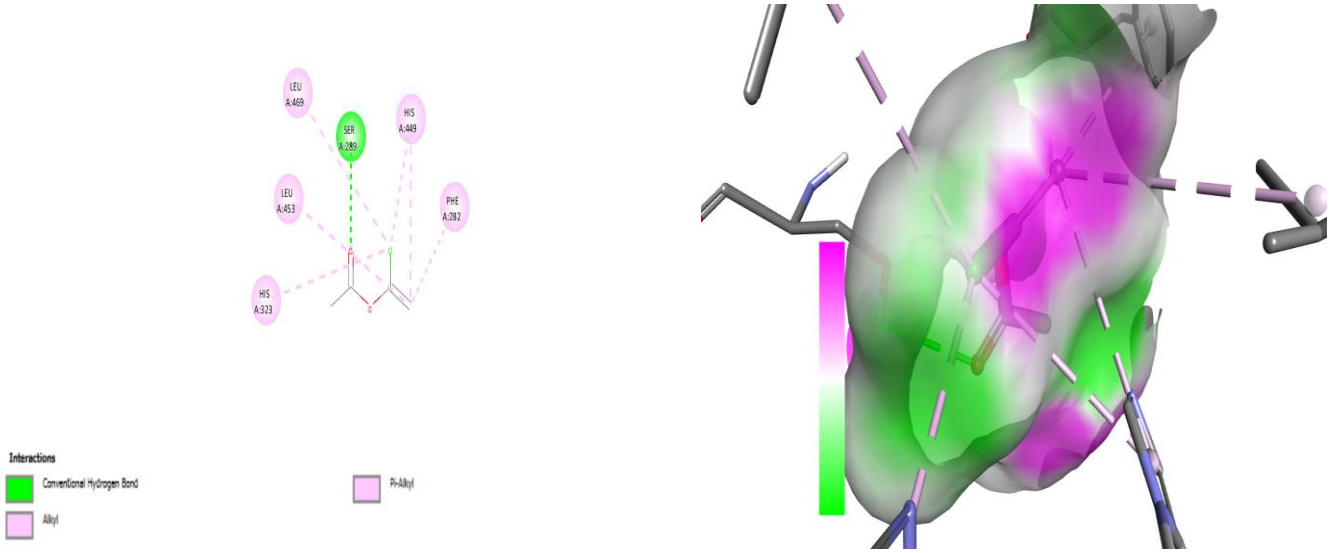

2D and 3D molecular interaction between oxalic acid, 2-ethyl hexyl isohexyl ester and PPAR gamma




2D and 3D molecular interaction between oxalic acid, isobutyl nonyl ester and PPAR gamma




2D and 3D molecular interaction between Terpinen-4-ol and PPAR gamma




2D and 3D molecular interaction between trans-2-chloro vinyl acetate

**DISCUSSION**

Molecular docking is an important tool largely used for computational drug design and discovery. Protein-ligand docking is employed in the prediction of the position and orientation of a ligand as it is bound to a protein receptor or enzyme. The receptor (protein) and ligand play an important role in structural based drug design. The target protein is peroxisome proliferator activated receptors gamma (PPAR-γ). Peroxisome proliferator activated receptors (PPARs) are nuclear receptors that participate in the transcriptional regulation of genes involved in lipid and carbohydrate metabolism and inflammation, particularly in obesity, hypercholesterolemia, insulin resistance and atherosclerotic conditions [15,16], they have received much attention from researchers due to their profound effect on glucose and lipid metabolism [15].

The characterization of the methanol leaf extract and the most potent fraction of *Napoleonae imperialis* were carried out by GC-MS which revealed the presence of 62 and 89 phytochemicals as shown in (Table 1 and Table 3). To examine the complete intermolecular relations between the ligand and the target protein (PPAR-γ). A computerized docking software AutoDock vina 4.2 was employed. It executes grid-based ligand docking with energetics and investigations for profitable connections between one or more characteristically little ligand compounds and a specific bigger receptor compound. Our findings showed that the methanol leaf extract contains 8 bioactive compounds, while the most potent fraction of the extract contains 17 bioactive compounds as shown in (Table 2 and Table 4) which serves as the ligands that demonstrated high binding affinities for the target protein (PPAR-γ). Three dimensional structural data on the target was obtained from the Protein Data Bank (PDB) entry 4EM9. The receptor preparation was executed to remove water molecules not related to the active spots and similarly to restore the natural position, and to aid the addition of hydrogen molecules. The bioactive compounds extracted from *Napoleonae imperialis* leaves obtained from GC-MS analysis were docked into the active site of PPAR-γ. A correlation was calculated by Glide score. The greatest correct technique of assessing the precision of a docking technique is to investigate how intimate the smallest energy level (binding conformation) is projected by the compound scoring activity. Three factors are generally examined when determing the outcomes. These are, Gscore, H-bond energy and residual interaction. This forms the basis in which the binding affinity of ligand towards the binding receptor is elucidated. The more negative the value of the standard free energy charge, the better the binding affinity of the ligand with receptor. Residual interaction shows where the ligand exactly binds to a particular amino acid of the protein [17]. The phytochemicals obtained from the leaves were analyzed by comparing their binding affinity to the receptor (Table 2 and Table 4). From the result, Dibutyl phthalate and 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione from methanol leaf extract of *Napoleonae imperialis* showed a high binding affinity of −7.3 kcal/mol as predicted by AutoDock/Vina, while Guaiol from the most potent fraction of the extract shows a high binding affinity of −7.5 kcal/mol. The result was confirmed by using Mcule which is an online drug discovery platform. From this result, Dibutyl phthalate, 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and Guaiol from *Napoleonae imperialis* leaves were identified as the lead compounds and thereby acts as a potent antidiabetic target. In order to confirm the potency of the lead compounds from the leaf with a standard drug, glibenclamide was docked with PPAR-γ using AutoDock/Vina. This gives a binding result of −10.7 kcal/mol with the binding pose [18].

Our in vitro experiments demonstrate that Dibutyl phthalate, 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and Guaiol from *Napoleonae imperialis* leaves binds 4EM9, and in itself activates its function and thus may act as an antidiabetic drug.

## CONCLUSION

The receptor (Protein) and ligand plays an important role in structural based drug design. In the present work, phytochemicals were obtained from the crude extract and fraction of *Napoleonae imperialis* by Gas Chromatography Mass spectrometry (GC-MS) analysis. The presence of different bioactive compounds provides evidence for the efficacious use this plant leaf for various ailments by traditional specialists. In this study, we docked the receptor PPAR-γ with the phytochemicals and from this, Dibutyl phthalate, 7,9-ditert-butyl-1-oxaspiro (4,5) deca,6,9-diene,2,8-dione and Guaiol from the crude extract and fraction holds a promising lead target formation against diabetes based on molecular docking analysis (minimum hydrogen bond length and maximum docked score). In-vivo and in-vitro approaches are therefore recommended to elucidate the molecular mechanism of these compounds to act as potent drug against type 2 diabetes.

## ACKNOWLDGEMENT

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## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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