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DETERMINATION OF THE ANTIBACTERIAL POTENCY OF OLEANOLIC ACID ISOLATED FROM *TRIFOLIUM PRETENSE*; THE MODIFICATION FOR THE IMPROVEMENT OF DRUGLIKENESS

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ABSTRACT

Background: *Furunculosis is highly contagious disease that affects fish of all ages. Also known as infection with* Aeromonas salmonicida*, the infection causes high mortality in salmonids, though some other species of fish are affected. The disease is one of the most commercially significant salmonid diseases, occurring in freshwater and marine salmonid aquaculture in all countries.* Aeromonas salmonicida *is a gram-negative bacillus that is critical to both wild and cultivated fish, especially salmon, because it is the causative agent of the disease furunculosis . Most strains of the bacterium are non-motile.* A. salmonicida *is a facultative aerobe, preferring to obtain energy through the utilization of oxygen as a terminal electron acceptor.The bacterium's optimal growth temperature is between 22 and 25°C. The maximum temperature that it can grow at is 34.5°C. Oleanolic acid is a pentacyclic triterpene that occurs widely in many plants as the free acid or the aglycone for many saponins. It is biosynthesized from lupane. It can rearrange to the isomer, ursolic acid, or be oxidized to taraxasterol and amyrin.*

Materials and Methods: *A molecular docking study was carried out on seven analogous structurally similar oleanolic acid against* Aeromonas salmonicida *cytochrome oxidase using the Autodock Vina software. An extensive study on the structure activity relationship was also carried out with these molecules. The physicochemical analysis, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of oleanolic acid and its analogues were evaluated. These molecules were designed by substituting the COOH group attached to the carbon-5 of oleanolic acid with C_2H_5, CH_3, $CONH_2$, NH_2, OCH_3 and HO groups. The scoring function (empirical binding free energy) was used to estimate the inhibitory activity of the protein-ligand complex. The Swiss Model server was used to build the 3D model of* Aeromonas salmonicida *cytochrome oxidase.*

Results: *The binding energy of oleanolic acid was -10.2Kcal/mol, while the free binding energies of the C_2H_5, CH_3, $CONH_2$, NH_2, OCH_3 and HO analogues of oleanolic acid were -9.5, -10.1, -9.1, -9.0, -9.4 and -9.8Kcal/mol respectively. All the modified analogues of oleanolic acid showed higher values than the oleanolic acid. These higher values (less negative values), means that oleanolic acid showed a better antibacterial activity than its analogues while the $CONH_2$ analogue showed improved druglikeness characteristics.*

Conclusion: *These results clearly indicated that the oleanolic acid may be a better antibacterial agent but the $CONH_2$ analogue being a better drug-like compound having improved on the gastrointestinal absorption rate exhibited by oleanolic acid and other modified analogues.*

KEYWORDS: *Docking; Oleanolic acid;* Aeromonas salmonicida *cytochrome oxidase; Pharmacokinetics; Lipophilicity.*

INTRODUCTION

Aeromonas salmonicida subsp. *salmonicida* causes severe septicemia and acute mortality in susceptible salmonid hosts [1]. The mode of infection, nature of pathology, and the degree of mortality, however, is interrelated with the quality of environmental parameters and furthermore affected by the age and innate resistance of the host [2]. Peracute infections most often occur in fingerling fish, which may darken in color and die without showing marked clinical indications of disease. Only a slight exophthalmia may be evident. Acute infections often occur in juvenile and adult fish that darken in color and hemorrhage at the base of fins and oral cavity. Internal hemorrhages may be evident in the abdominal walls, viscera, and heart of affected fish [3]. The spleen is enlarged, and the liver can have subcapsular hemorrhages, or focal necrosis of parenchymatous tissue [4]. Affected fish may display erratic swimming behavior, become sluggish, and stop feeding. Consequently, the stomach and intestine are usually devoid of food, and the lumen may contain sloughed epithelial cells, mucus, and blood. The reproductive organs are commonly hemorrhaged and the intestine is often severely congested [5]. The chronic form of furunculosis usually occurs in older fish that have become more refractive to the disease or among species that have greater innate resistance to infection by A. salmonicida. One or more furuncle-like lesions may be present on the dermis and ulcers may extend deep into the musculature. Internally, chronically infected salmonids show a general visceral congestion and peritonitis. Hemorrhages may occur over the pyloric area and liver, and kidneys are soft or friable [6].

The development of the characteristic "furunclelike" lesion is not a consistent finding, but is most often associated with chronic infections [7]. When these lesions are present, they consist of tissue fluid exudate, necrotic tissue, and some macrophages [8]. Thus, the furunculosis lesion differs from the true furuncle associated with homeothermic vertebrates, which is characterized by a necrotic mass of polymorphonuclear leukocytes. Degeneration of myofibrils, fragmentation of muscle fibers, and hemorrhage of the entire muscular tissue is evident within the swelling lesion and leads to a colliquative necrosis of the musculature in the most serious lesions [9]. Bacteria may also colonize the gill epithelium on or between the secondary lamellae [10] where they may be enclosed within a membrane that is continuous with the basement membrane of the lamellar epithelium [11]. Bacterial embolisms may develop in gill lamellae causing a further proliferation of branchial epithelial cells and a subsequent fusion of gill lamellae that impairs circulation [12].

Oleanolic acid can be found in clove, olive oil, *Phytolacca americana* (American pokeweed), and *Syzygium* spp, garlic, etc. It was first studied and isolated from several plants, including *Olea europaea* [13] (leaves, fruit), *Rosa woodsii* (leaves), *Prosopis glandulosa* (leaves and twigs), *Phoradendron juniperinum* (whole plant), *Syzygium claviflorum* (leaves), *Hyptis capitata* (whole plant), *Mirabilis jalapa* [13] and *Ternstroemia gymnanthera* (aerial part). Other *Syzygium* species including java apple (*Syzygium samarangense*) and rose apples contain it. Oleanolic acid is relatively non-toxic, hepatoprotective,

andexhibits antitumor,antibacterial and antiviral properties [14]. Oleanolic acid was found to exhibit weak anti-HIV [15] and weak anti-HCV activities *in vitro*, but more potent synthetic analogs are being investigated as potential drugs [16].

The aim of this study is to determine the potency of oleanolic acid by docking the compound against *Aeromonas salmonicida* cytochrome oxidase and also to modify the compound in order to improve the its druglikeness.

Figure 1: Brook trout (Salvelinus fontinalis) showing a furuncle like lesion near its dorsal fin caused by infection with Aeromonas salmonicida subsp. salmonicida (Cipriano, 1997).

MATERIALS AND METHODS

Sequence Retrieval

The *Aeromonas salmonicida* cytochrome oxidase amino acid sequence was obtained from the National Center for Biotechnological Information database (NCBI) [18]. The protein is assigned an accession number KFN19471.1

Protein Preparation

The 3D structure of *Aeromonas salmonicida* cytochrome oxidase was modeled using the Swiss model server [2]. The template with a sequence identity of 49.20% and a resolution of 2.0(Å) was selected to build the 3D model of the enzyme.

Designing of Oleanolic Acid Structural Analogues

The structure of oleanolic acid (Figure 2) was drawn with the Marvin Sketch software [20]. The structural analogues of oleanolic acid were developed with structural modifications and the addition of different substituents [21]. The COOH group attached to the carbon-5 of oleanolic acid was substituted with C_2H_5, CH_3, $CONH_2$, NH_2, OCH_3 and HO groups. The structures were built with the Marvin Sketch software and minimized using the Chimera software [22].

Molecular docking

Molecular docking was performed using the AutoDock Vina Software [23]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of oleanolic acid and its analogues were determined using SwissADME Server [24].

RESULTS AND DISCUSSION

Chemical Structures

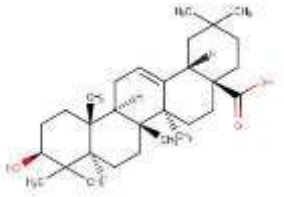

Fig 2: Oleanolic acid

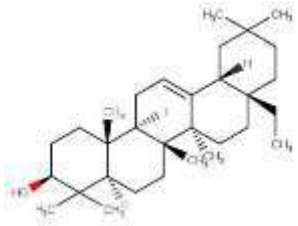

Fig 3: C_2H_5 analogue of oleanolic acid

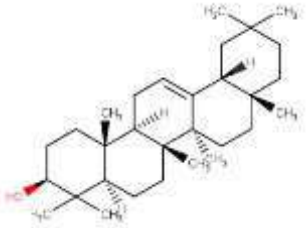

Fig 4: CH_3 analogue of oleanolic acid

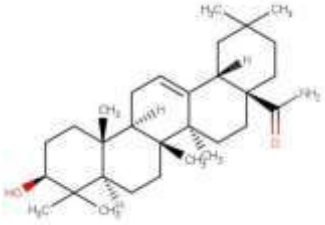

Fig 5: $CONH_2$ analogue of oleanolic acid

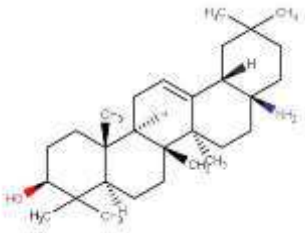

Fig 6: NH_2 analogue of oleanolic acid

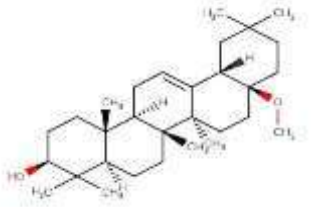

Fig 7: OCH_3 analogue of oleanolic acid

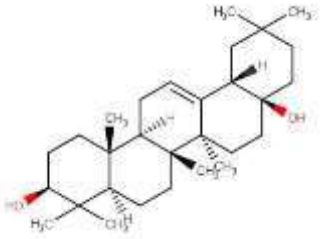

Fig 8: HO analogue of oleanolic acid

In-Silico Pharmacokinetics

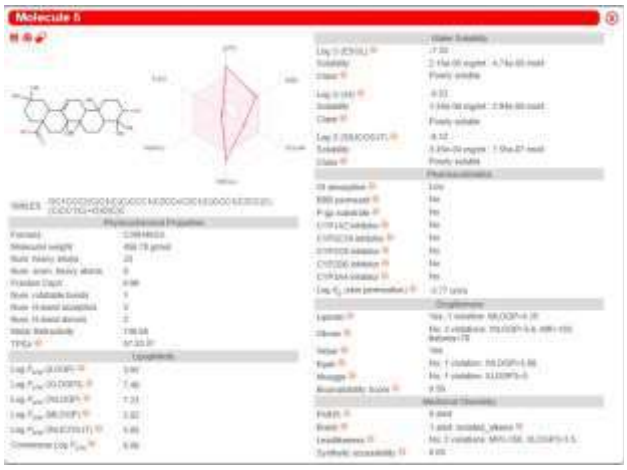

Fig 9: Oleanolic acid

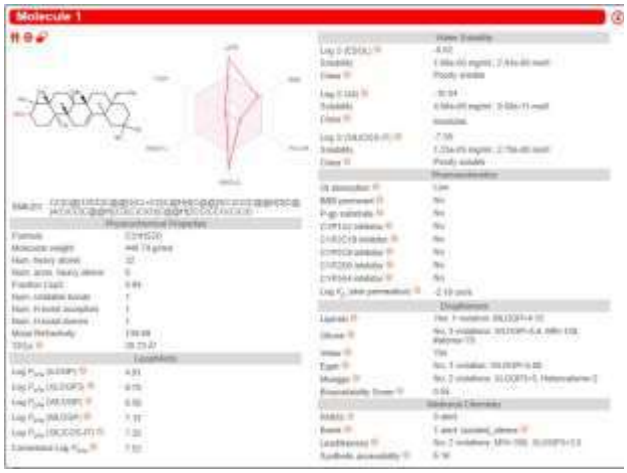

Fig 10: C_2H_5 analogue of oleanolic acid

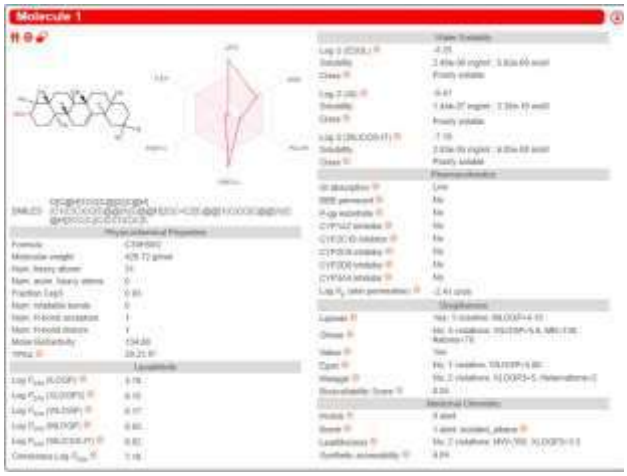

Fig 11: CH_3 analogue of oleanolic acid

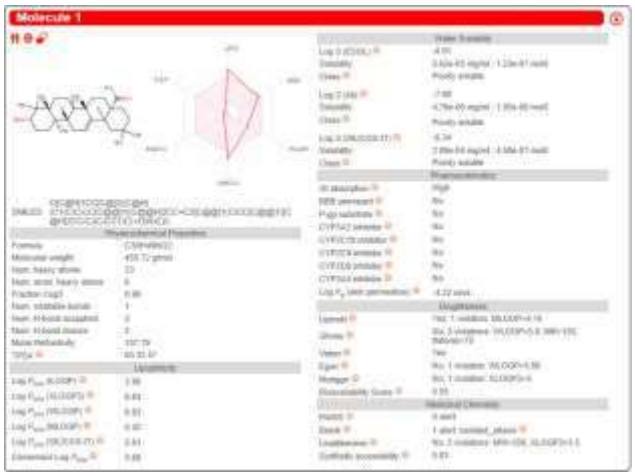

Fig 12: $CONH_2$ analogue of oleanolic acid

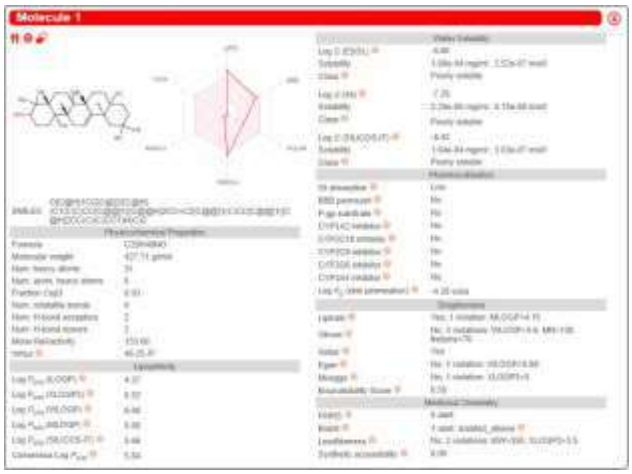

Fig 13: NH_2 analogue of oleanolic acid

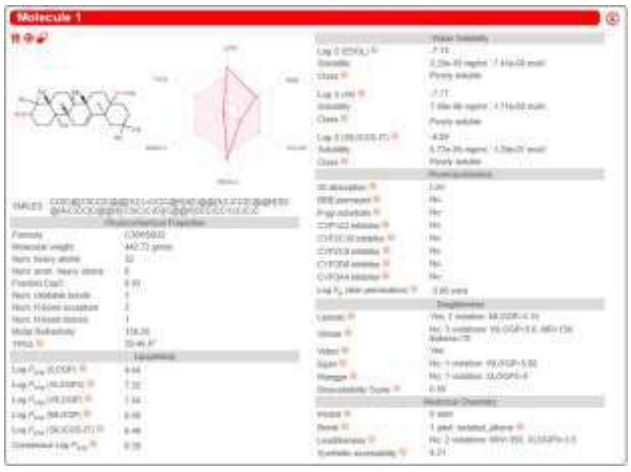

Fig 14: OCH_3 analogue of oleanolic acid

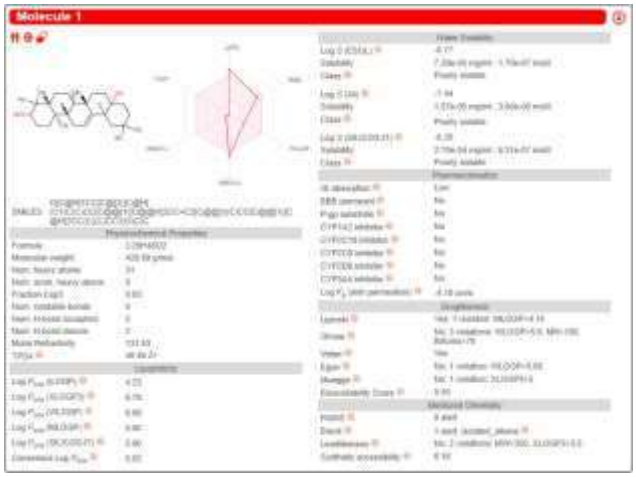

Fig 15: HO analogue of oleanolic acid

MOLECULAR DOCKING RESULTS

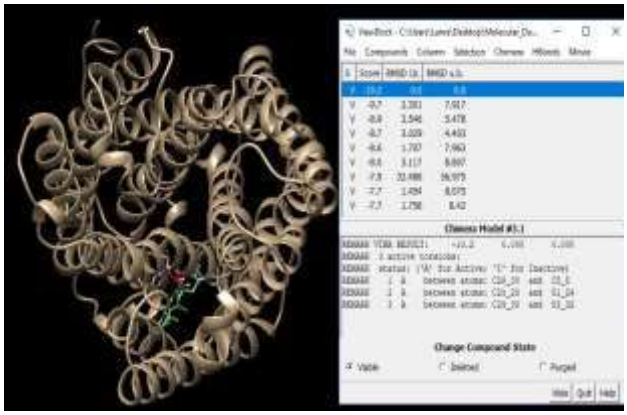

Fig 16: Oleanolic acid in complex with *A. salmonicida* cytochrome oxidase

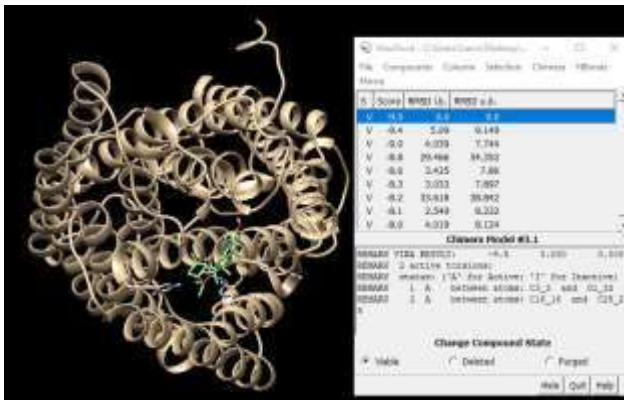

Fig 17: C_2H_5 analogue in complex with *A. salmonicida* cytochrome oxidase

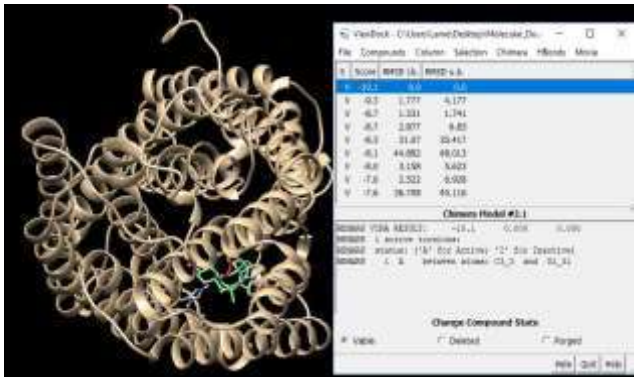

Fig 18: CH_3 analogue in complex with *A. salmonicida* cytochrome oxidase

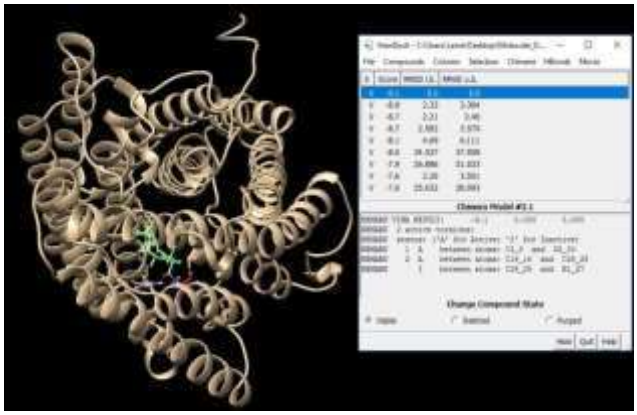

Fig 19: $CONH_2$ analogue in complex with *A. salmonicida* cytochrome oxidase

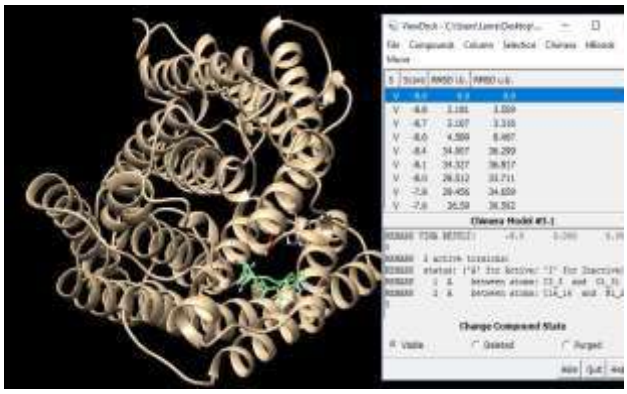

Fig 20: NH_2 analogue in complex with *A. salmonicida* cytochrome oxidase

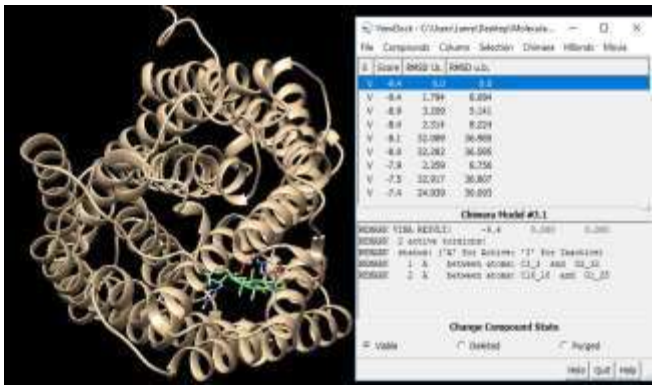

Fig 21: OCH_3 analogue in complex with *A. salmonicida* cytochrome oxidase

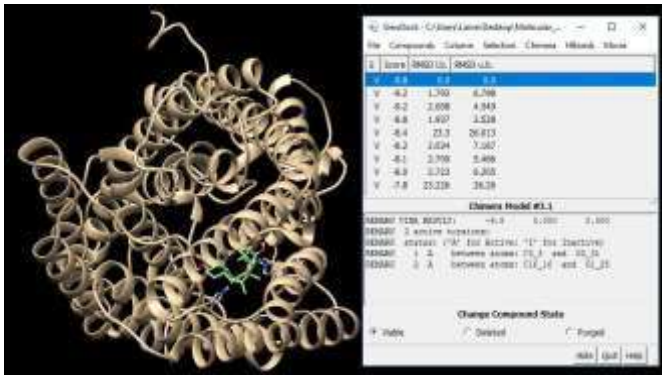

Fig 22: HO analogue in complex with *A. salmonicida* cytochrome oxidase

The docking poses of all the compounds showed that they bind in a very similar pattern with the active site of *Aeromonas salmonicida* cytochrome oxidase, as is evident from the superposition of the oleanolic acid and all its 6 analogues in Figures 12-22. The interaction between oleanolic acid and the different monosubstituted analogues with *Aeromonas salmonicida* cytochrome oxidase shows steric interactions with the amino acid residues. The calculated free energy of binding of the falcarindiol and its analogues were -9.5, -10.1, -9.1, -9.0, -9.4 and -9.8Kcal/mol respectively. This confirms that the structural modification implemented in this study is significantly related to their activity [25, 26]. Also, this proved the reliability of the docking results [27].

The solubility of a compound in water could improve its biotransformation and elimination as a drug [28]. Oleanolic acid and all the modified analogues were soluble in water

The molecular weight of all the substituted derivatives including oleanolic acid were less than 500g/mol, showing that they can be considered as drugs [29]. A compound can also be considered drug-like if it is characterized by high lipophilicity (less than 5) [30]. This is expressed as Log Po/w. The lipophilicity values of oleanolic acid and all the modified compounds analogues are higher than 5. This implies that major modifications must be effected on the compounds for them to be drug-like in this regard.

Lipinski's rule of 5 [31] helps in distinguishing between drug-like and non drug-like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules: Molecular mass less than 500g/mol; High lipophilicity (expressed as Log Po/w less than 5); Less than 5 hydrogen bond donors; Less than 10 hydrogen bond acceptors; Molar refractivity should be between 40-130. These filters help in early preclinical development and could help avoid costly late-stage preclinical and clinical failures [32]. Oleanolic acid and all its modified analogues complied with a minimum of two of the Lipinski's rule and therefore are likely to be drugs in this regard.

High penetration is needed for most of the drugs targeting the central nervous system (CNS), whereas blood brain barrier (BBB) penetration should be minimized for non-CNS drugs to avoid undesired side-effects [33]. Pharmacokinetically, only the

$CONH_2$ analogue of oleanolic acid exhibited a high rate the gastrointestinal drug absorption and could not permeate the blood brain barrier (BBB). This makes it safe for administration.

For synthetic accessibility, values of 5 to10 means that the drug could be synthesized [32]. Oleanolic acid and all its modified analogues showed values that ranges between 5 to 7. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by pre- clinical studies are further recommended.

CONCLUSION

An In-Silico Structure Activity Relationship and molecular docking experiment was carried out on *Aeromonas salmonicida* cytochrome oxidase, using oleanolic acid and six of its structurally similar analogues as the experimental compounds. The results obtained indicated that all the analogues may have a good antibacterial activity having shown a high binding energy value and exhibited a high level of specificity and affinity with the target enzyme. The $CONH_2$ analogue of oleanolic acid showed an improvement in the druglikeness attributes as it appeared to be the only compound that exhibited a high gastrointestinal absorption rate.

Oleanolic acid and all its analogues can pose no threat to the Central Nervous System (CNS) as they cannot penetrate the blood brain barrier. This means that the administration of these drugs cannot produce any undesirable side effect. The laboratory synthesis and pre-clinical studies on the $CONH_2$ modified derivative of oleanolic acid with *Aeromonas salmonicida* cytochrome oxidase is therefore recommended.

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