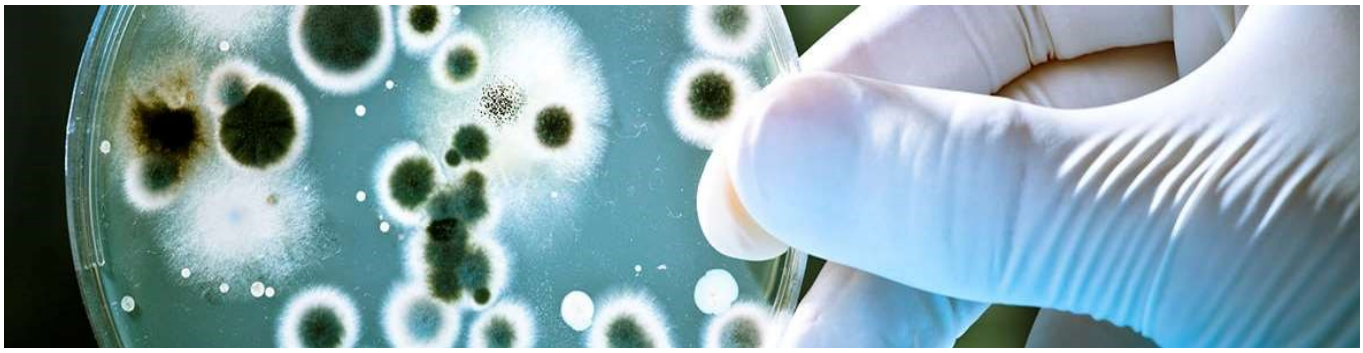
Drug Target Identification and Inhibition of *Aeromonas salmonicida* Cytochrome Oxidase in the Treatment of Furunculosis Infected *Clarias gariepnus*



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ABSTRACT

Background: Fishes are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing diseases are considered to be saprophytic in nature. These bacteria only become pathogens when fishes are physiologically unbalanced, nutritionally deficient, or there are other stressors, i.e., poor water quality, overstocking, which allow opportunistic bacterial infections to proceed. Furuncles classically are described as a dark, raised tumefaction involving the skin, subcutis and underlying skeletal musculature. These lesions will ulcerate and drain a serosanguinous fluid. These lesions develop from the localization of *Aeromonas salmonicida* bacteria in the muscle or skin. The lesion histologically is characterized by marked necrosis of the skin, subcutis and skeletal muscle with mild to minimal acute inflammatory infiltrates in the acute stage.

Materials and Methods: A molecular docking study was carried out on five structurally diverse isolated clover compounds against *Aeromonas salmonicida* cytochrome oxidase, using the Autodock Vina software. The Swiss Model server was utilized in the 3D structure modelling of the cytochrome oxidase enzyme and extensive structure activity relationship study was also carried out on these molecules using the SwissADME. The isolated compounds from clover were designed using the ChemAxon software. The scoring function (empirical binding free energy) was used in the estimation of the inhibitory activity of the protein-ligand complex.

Results: The binding energy of acetyleugenol, furfural, gallic acid, methyl-n-amyl ketone, oleanolic acid, rhamnetin, sesquiterpene lactone and vanillin were -6.8, -4.6, -6.1, -4.6, -10.2, -9.4, -8.4 and -5.7Kcal/mol respectively. The low values (negative) of free binding energies displayed by the bioactive components of clover means that they show a high level of antibacterial activity. All compounds except the oleanolic acid violated none of the lipinski's rule of 5 which makes them druglike.

Conclusion: The results from the in-silico pharmacokinetics and molecular docking clearly indicated that the rhamnetin substituent may be a better antibacterial agent, having exhibited a good binding affinity with the *Aeromonas salmonicida* cytochrome oxidase and also showed good druglikeness characteristics. Laboratory synthesis and pre-clinical studies of the rhamnetin component with *Aeromonas salmonicida* cytochrome oxidase is recommended in order to confirm its potentials as a better antibacterial agent than the other compounds isolated from clover.

KEYWORDS:

Bacteria; Furuncules; Cytochrome oxidase; Molecular docking; Oleanolic acid

I. INTRODUCTION

Clarias gariepinus is a species of catfish of the family Clariidae, the air breathing catfishes. It is a large, eel-like fish, usually of dark gray or black coloration on the back, fading to a white belly. In Africa, this catfish has been reported as being second in size only to the vundu of the Zambesian waters [1] although FishBase suggests the African sharptooth catfish surpasses that species in both maximum length and weight [2, 3].

Aeromonas salmonicida is a pathogenic bacterium that severely impacts salmonid populations and other species. It was first discovered in a Bavarian brown trout hatchery by Emmerich and Weibel in 1894 [4]. *Aeromonas salmonicida's* ability to infect a variety of hosts, multiply, and adapt, make it a prime virulent bacterium [5]. *A. salmonicida* is an etiological agent for furunculosis, a disease that causes septicemia, haemorrhages, muscle lesions, inflammation of the lower intestine, spleen enlargement, and death in freshwater fish populations. It is found worldwide with the exception of South America [4]. The major route of contamination is poor water quality; however, it can also be associated stress factors such as overcrowding, high temperatures, and trauma. Spawning and smolting fish are prime victims of furunculosis due to their immunocompromised state of being [5]. *Aeromonas salmonicida* is a Gram-negative, facultatively anaerobic, nonmotile bacterium. It is rod-shaped, about 1.3–2.0 by 0.8–1.3 µm in size, and grows optimally at temperatures between 22 and 25 °C [4]. The bacterium readily ferments and oxidizes glucose, and is catalase-positive and cytochrome oxidase-positive. Its molecular properties include a special surface protein array called the A-layer, which is believed to be responsible for the bacterium's virulent traits, and lipopolysaccharide, the cells' major cell envelope antigen [6].

Furunculosis was reported for the first time as early as in 1894 in Germany [7]. The name furunculosis was given because the diseased fish had furuncle-like swellings, which were ulcerative at a later stage of the disease. In the earlier literature the causative agent was referred to as Bacterium or Bacillus salmonicida [8], but it was later named *Aeromonas salmonicida* by [9]. Isolates of the bacterium initially appeared to be very homogeneous, but from the 1960s an increasing number of studies reported isolates with properties differing from those of the typical strains of *A. salmonicida* [10]. McCarthy (1977) classified *A. salmonicida* into two groups comprising typical and atypical strains [12].

Clove (*Trifolium pratense*) has been used for its antiseptic and antibacterial effects and has been studied for use as an anticoagulant and anti-inflammatory effects [13]. Clove buds yield approximately 15% to 20% of a volatile oil that is responsible for the characteristic smell and flavor [14]. The bud also contains a tannin complex, a gum and resin, and a number of glucosides of sterols. The principal constituent of distilled clove bud oil (60% to 90%) is eugenol (4-allyl-2-methoxyphenol) [15]. The oil also contains about 10% acetyleugenol and small quantities of gallic acid, sesquiterpenes, furfural, vanillin, and methyl-n-amyl ketone. Other constituents include flavonoids, carbohydrates, lipids, oleanolic acid, rhamnetin, and vitamins [16].

This study is aimed at determining the efficacy of compounds isolated from clove in the treatment of *Aeromonas salmonicida* linked furunculosis of *Clarias gariepinus* through the in-silico pharmacokinetics and molecular docking approach.

II. MATERIALS AND METHODS

Sequence retrieval

The *Aeromonas salmonicida* cytochrome oxidase amino acid sequence was obtained from the National Center for Biotechnological Information database (NCBI) [17]. The protein was assigned an accession number of KFN19471.1.

Protein 3D Structure Modelling

The 3D structure modeling of the protein was achieved using the Swiss Model server [18].

Physiological–biochemical characterization

The Expasy Protparam server [19] was used for the physicochemical characterization and to know the molecular weight, theoretical isoelectric point (pI), total number of negative and positive residues, aliphatic index, extinction coefficient, instability index, and grand average hydropathicity (GRAVY) of *Aeromonas salmonicida* cytochrome oxidase [20].

3D Structure of Isolated Compounds

The 3D structure of the isolated compounds from clover were built by inputting the simplified molecular-input line-entry system (SMILES) for each compound into the "Build structure" interface of Chimera [21].

Molecular docking

The docking was performed using the AutoDock Vina Software [22]. Physicochemical, lipophilicity, solubility, pharmacokinetics and Lipinski druglikeness of the isolated compounds were determined using the SwissADME Server [23].

III. RESULTS AND DISCUSSION

Aeromonas salmonicida Cytochrome Oxidase Amino Acid Sequence (FASTA)

>KFN19471.1 cytochrome oxidase subunit I [Aeromonas salmonicida]
MIETLHAGHSHGPAAGLRRWLYTTNHKDIGTLYLLFALT
MFFTGGTMAMVIRAELFQPGLQLVEPLFFNQ
MTTVHGLVMVFGAVMPAFTGLANWLIPMMIGAPDMALP
RMNNWSFWILPFAFLILLSSLFMDGGGPSSGW
TFYAPLSTKYSGNSTALFVFAIHIMGISSIMGAINVIVTIFN
LRAPGMTWMKMPLFVWTWLITAFLLIAV
MPVLAAAVTMVLTDKYFGTSFFDAAGGGDPVMFQHIFW
FFGHPEVYIMILPAFGIVSSIIPTFSRKKLFG
YRSMVYATSSIAILSFLVWAHHMFTTGLPVVAELFFMYA
TMLIAVPTGVKVFNWVATMWRGSLTFETPML
FAIAFITLFTIGGFSGLMLAMIPADFQYHDTYFVVAHFHY
VLVTGAVFSILAAAYYWLPKWTGHMYDERL
GQWHFWCSLISVNVLFFPMHFVGLAGMPRRIPDYALQFA
DFNALISVGGFAFGLSQLLFVWGVIKCIRGG
NKATAQVWEGAEGLEWTLPSPPPYHSFQSPPDIK

3D Structure Modeling

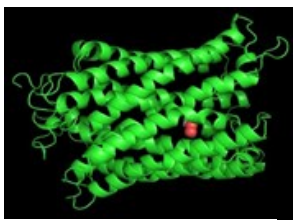

Fig 1: *Aeromonas salmonicida* Cytochrome Oxidase

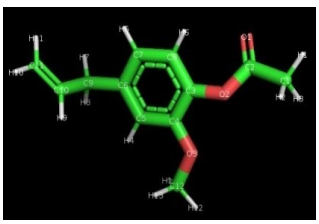

Fig 2: Acetyleugenol

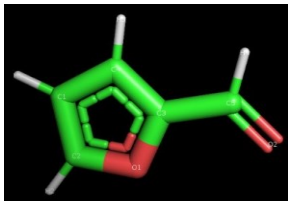

Fig 3: Furfural

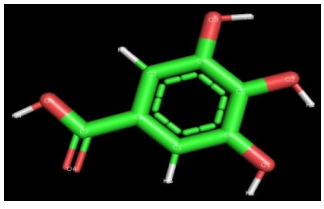

Fig 4: Gallic acid

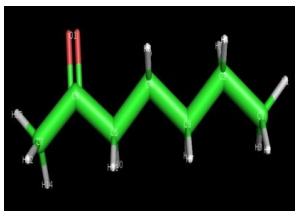

Fig 5:Methyl-n-amyl ketone

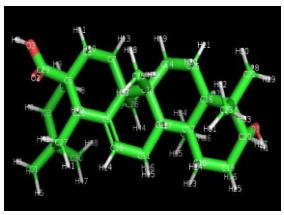

Fig 6: Oleanolic acid

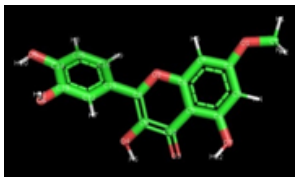

Fig7: Rhamnetin

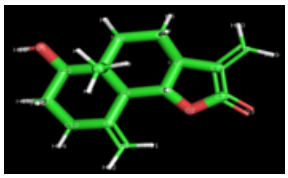

Fig 8: Sesquiterpene lactone

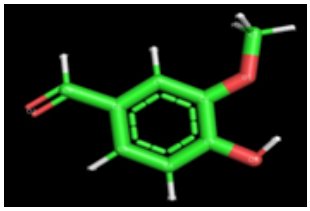

Fig 9: Vanillin

In-Silico Structure Activity Relationship

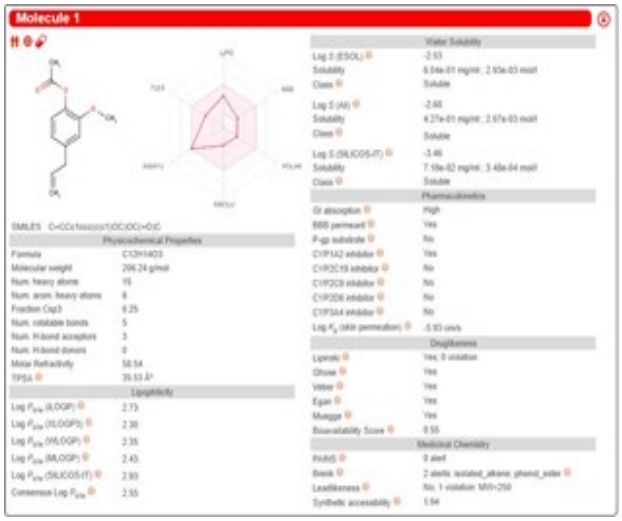

Fig 10: Acetyleugenol

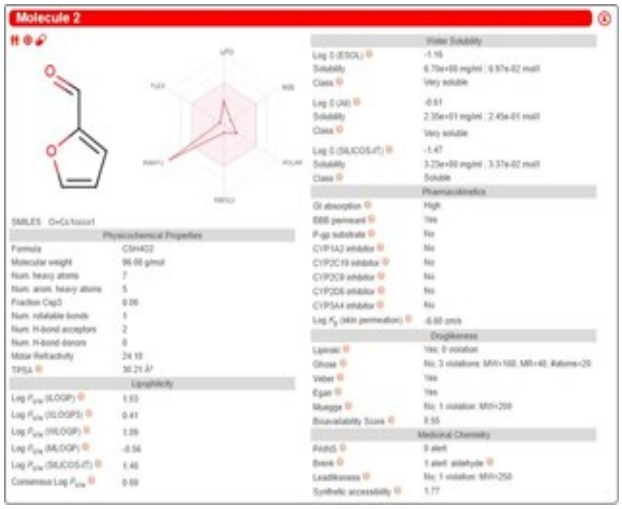

Fig 11: Furfural

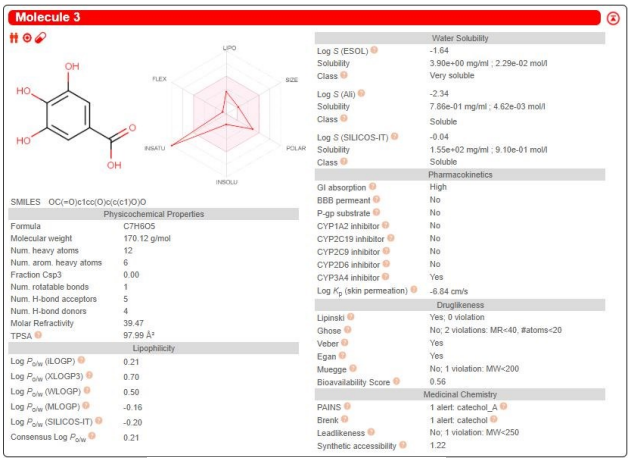

Fig 12: Gallic acid

Fig 13: Methyl-n-amyl ketone

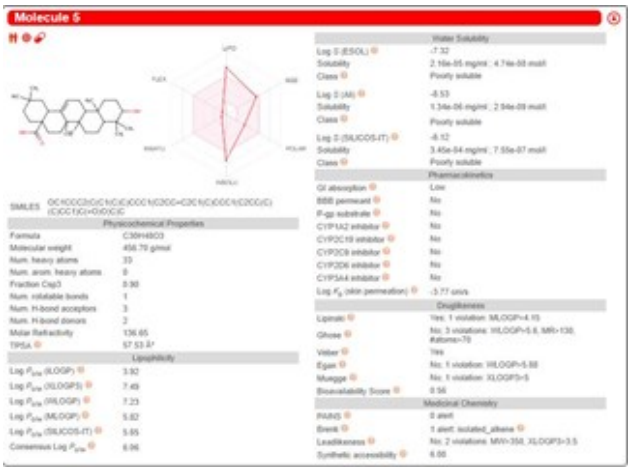

Fig 14: Oleanolic acid

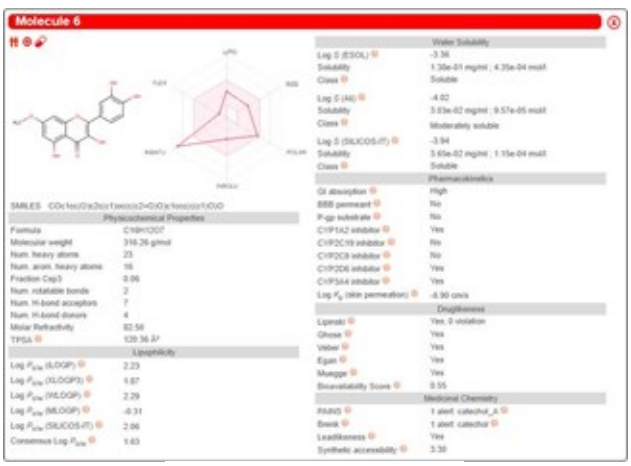

Fig 15: Rhamnetin

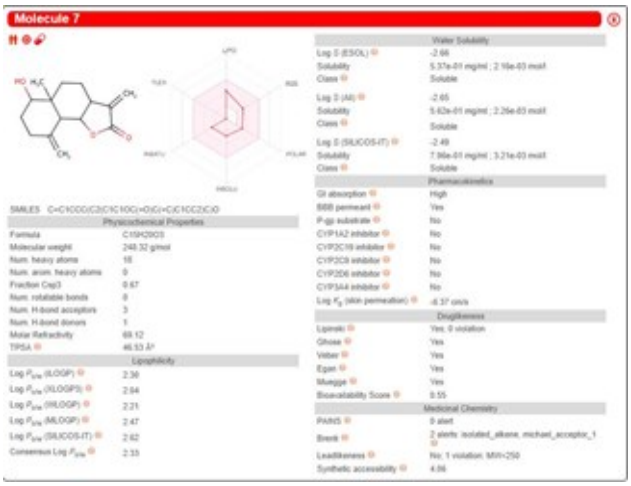

Fig 16: Sesquiterpene lactone

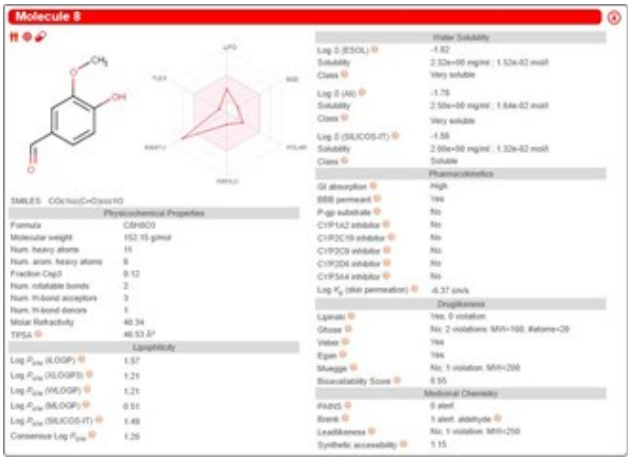

Fig 17: Vanillin

Docking of Isolated Compounds against Aeromonas salmonicida Cytochrome Oxidase

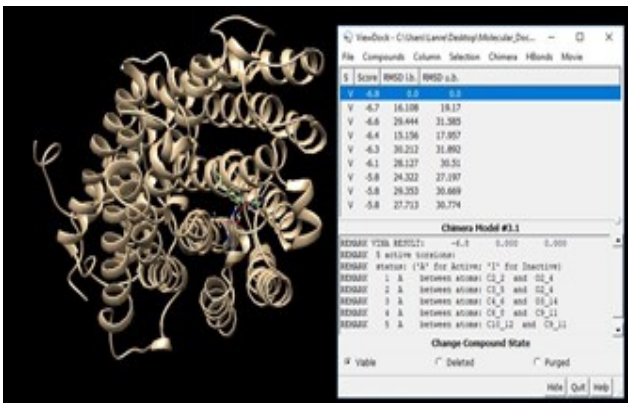

Fig 18: Acetyleugenol

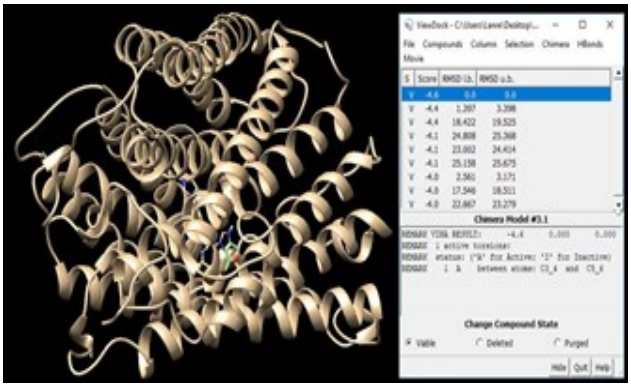

Fig 19: Furfural

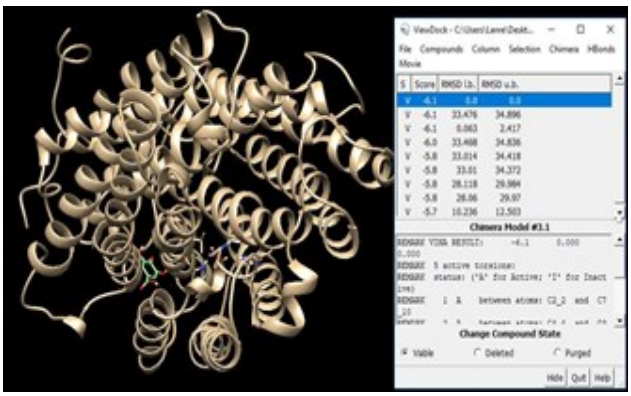

Fig 20: Gallic acid

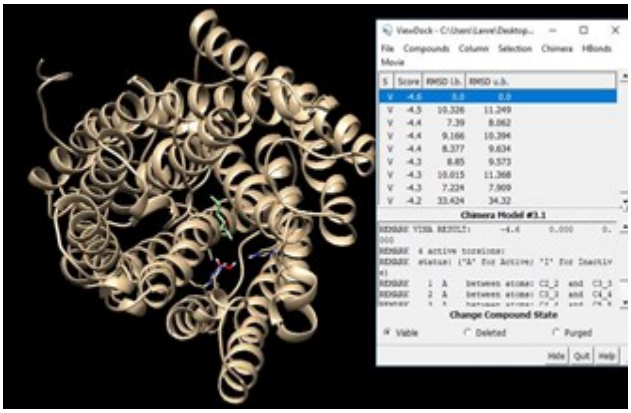

Fig 21: Methyl-n-amyl ketone

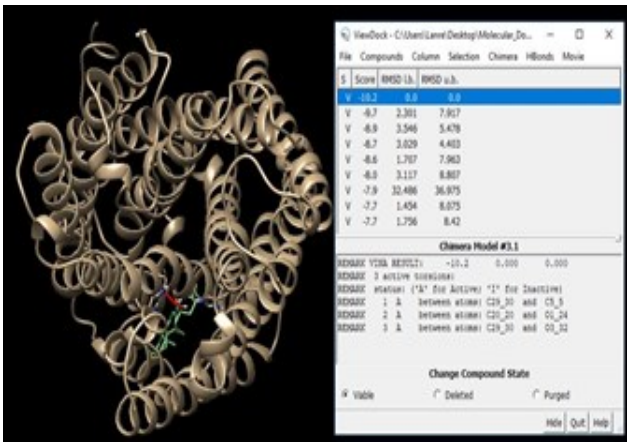

Fig 22: Oleanolic acid

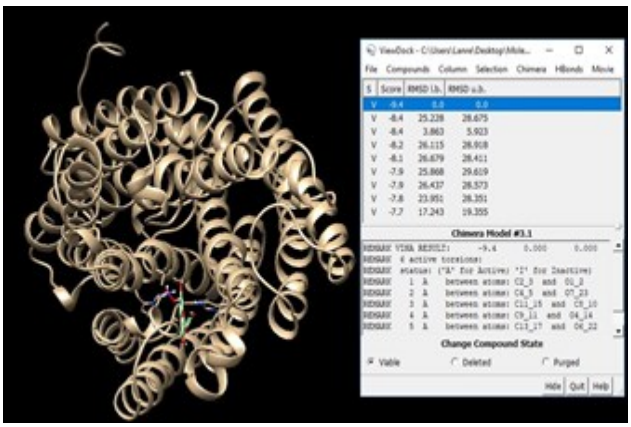

Fig 23: Rhamnetin

Fig 24: Sesquiterpene lactone

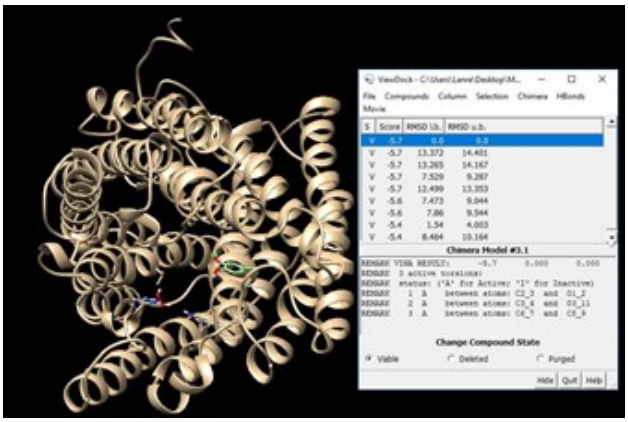

Fig 25: Vanillin

The pI of the *Aeromonas salmonicida* cytochrome oxidase going by the biochemical characterization analysis has predicted the protein to be slightly alkaline with a value of 7.87 [24]. The hydrophobicity scale produced values that define relative hydrophobicity of amino acid residues. The more positive the value, the more hydrophobic the amino acids located in that region of the protein [25]. The GRAVY calculator used in predicting the hydrophobicity assigned to the protein a value of 0.698.

The instability index provides an estimate of the stability of a protein in a test tube. A protein whose instability index is greater than 40 is predicted as unstable and a value below 40 predicts the protein may be stable [25]. The *Aeromonas salmonicida* cytochrome oxidase is therefore a stable protein with an instability value of 38.04.

Aeromonas salmonicida cytochrome oxidase contains 524 amino acid residues with a molecular weight of 58517.29. The docking pose exhibited by all the experimental compounds showed that their binding pattern with the active site of *Aeromonas salmonicida* cytochrome oxidase is in a similar orientation, as is evident from the superposition of the compounds in Figure 18-25. The compounds also show a steric interaction with the amino acid residues of *Aeromonas salmonicida* cytochrome oxidase. The calculated free energy of the compounds isolated from clover (acetyleugenol, furfural, gallic acid, methyl-n-amyl ketone, oleanolic acid, rhamnetin, sesquiterpene lactone and vanillin) were -6.8, -4.6, -6.1, -4.6, -10.2, -9.4, -8.4 and -5.7Kcal/mol respectively. This proved the reliability of the docking results going by the structural similarities exhibited by the compounds [26].

The biotransformation and elimination of a druglike compound is a function of its solubility [27]. All the experimental compounds were soluble in water except oleanolic acid which appeared to be poorly soluble. This makes them likely to be drugs (figure 10-17).

A compound can be considered drug-like if it is characterized by high lipophilicity (less than 5) [28] and low molecular weight (less than 500g/mol) [29]. Lipophilicity is expressed as Log Po/w. The lipophilicity value of all the experimental compounds except Oleanolic acid are less than 5 and are most likely to be drugs (figure 10-17).

Distinguishing between drug-like and non drug-like molecules requires the application of the lipinski's rule of 5 [30]. The probability of success or failure is dependent on the compliance 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. For the avoidance of costly late-stage preclinical and clinical failures, application of these filters could help in the early stages of the preclinical drug development [31]. Only oleanolic acid violated the lipinski's rule. Other experimental compounds violated none of the lipinski's rule of five and therefore are likely to be drugs (figure 10-17).

Drugs targeting the central nervous system (CNS) must have a characteristic high penetration rate, whereas penetration of the blood brain barrier (BBB) should be limited for non-CNS drugs to avoid undesired side-effects [32]. The gastrointestinal drug absorption of all the compounds but oleanolic acid was high and only gallic acid, oleanolic acid and rhamnetin showed no ability to cross the blood brain barrier (BBB). These three therefore pose no threat to the CNS.

For synthetic accessibility, values of 1 to 5 means that the drug could easily be synthesized [31]. All the experimental compounds but oleanolic acid showed values less than 5. This means that the compounds can easily be synthesized in the laboratory. Synthetic studies followed by preclinical test of the compounds are further recommended.

IV. CONCLUSION

An In-Silico structure activity relationship and molecular docking study was carried out on isolated compounds from clover (*Trifolium pratense*) against *Aeromonas salmonicida* cytochrome oxidase. The obtained result showed that the rhamnetin component showed the best therapeutic prospect against the bacterial enzyme as it is characterized by a high binding energy of -9.4Kcal/mol and exhibited good pharmacokinetics characterstics.

We recommend the laboratory synthesis of rhamnetin for further preclinical trial against the *Aeromonas salmonicida* cytochrome oxidase.

REFERENCES

[1]. **Anoop KR, Sundar KSG, Khan BA & Lal S (2009)** Common Moorhen Gallinula chloropus in the diet of the African catfish Clarias gariepinus in Keoladeo Ghana National Park, India. Indian Birds 5(2):22-23.

[2]. **Jansen van Rensburg, C., van As, J.G. & King, P.H. 2013.** New records of digenean parasites of Clarias gariepinus (Pisces: Clariidae) from the Okavango Delta, Botswana, with description of Thaparotrema botswanensis sp. n. (Plathelminthes: Trematoda). African Invertebrates 54 (2): 431–446.[1]

[3]. **Froese, Rainer and Pauly, Daniel, eds. (2014).** "Clarias gariepinus" in FishBase. March 2014 version.

[4]. **"Furunculosis".** Merck. Archived from the original on 30 July 2015. Retrieved 2011-06-11.

[5]. **Reith M, Singh R, Curtis B, Boyd J, & Bouevitch A. (2008).** The genome of Aeromonas salmonicida subsp. salmonicida A449: insights into the evolution of a fish pathogen. BMC Genomics. 9 (427), 1-15".

[6]. **Chart H, Shaw D, Ishguro E, &Trust T . (1984).** Structural and Immunochemical Homogeneity of Aeromonas salmonicida Lipopolysaccharide. Journal of Bacteriology. 158 (1), 16-22".

[7]. **Emmerich R, Weibel E (1894)** Ueber eine durch Bacterien erzeugte Seuche unter den Forellen. Archiv für Hygiene 21:1-21.

[8]. **McCraw BM (1952)** Furunculosis of fish. US Fish Wildl.Serv., Special scientific report: Fisheries No 84:1-87.

[9]. **Griffiths SG, Olivier G, Fildes J, Lynch WH (1991)** Comparison of western blot, direct fluorescent antibody and drop plate culture methods for the detection of Renibacterium salmoninarum in Atlantic salmon (Salmo salar L.). Aquaculture 97:117-129.

[10]. **Smith IW (1963)** The classification of "Bacterium salmonicida". J.Gen.Microbiol. 33:263-274.

[11]. **McCarthy DH (1977a)** Some ecological aspects of the bacterial fish pathogen - Aeromonas salmonicida. In: Skinner FA, Shewan JM (eds). Aquatic Microbiology. Soc.Appl.Bacteriol. Symp.Ser.6, pp 299-324. Academic Press, London.

[12]. **McCarthy DH (1977b)** The identification and significance of atypical strains of Aeromonas salmonicida. Bull.Off.int.Epiz. 87:459-463.

[13]. **Apatzidou DA, Riggio MP, Kinane DF.** Quadrant root planing versus same day full mouth root planing II. Microbiological findings. J Clin Periodontol 2004; 31:141-8.

[14]. **Apatzidou DA, Kinane DF.** Nonsurgical mechanical treatment strategies for periodontal disease. Dent Clin North Am 2010; 54:1-12

[15]. **Kumar P, Ansari SH, Ali J.** Herbal remedies for the treatment of periodontal disease--a patent review. Recent Pat Drug Deliv Formul 2009; 3:221-8.

[16]. **Newman DJ, Cragg DM.** Natural products as sources of new drugs over the last 25 years. J Nat Prod 2007; 70:461-77.

[17]. http://www.ncbi.nlm.nih.gov/

[18]. **Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T.** SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46(W1), W296-W303 (2018).

[19]. http://us.expasy.org/tools/protparam

[20]. **Gasteiger E., Hoogland C., Gattiker A., Duvaud S., Wilkins M.R., Appel R.D., Bairoch A.;** Protein Identification and Analysis Tools on the ExPASy Server; (In) John M. Walker (ed): The Proteomics Protocols Handbook, Humana Press (2005). pp. 571-607.

[21]. **Swanson, Richard Pommier (2004).** "The Entrance of Informatics into Combinatorial Chemistry". In Rayward, W. [Warden] Boyd; Bowden, Mary Ellen. The History and Heritage of Scientific and Technological Information Systems: Proceedings of the 2002 Conference of the American Society of Information Science and Technology and the Chemical Heritage Foundation. Medford, NJ: Information Today. p. 205. ISBN 1-57387-229-6.

[22]. **Trott, O.; Olson, A.J. (2010),** "AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading", Journal of Computational Chemistry, 31 (2): 455–461, doi:10.1002/jcc.21334.

[23]. **Daina A, Michielin O, Zoete V (2017)** A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7: 42717.

[24]. **Shi Q, Zhou Y, Sun Y.** Influence of pH and ionic strength on the steric mass-action model parameters around the isoelectric point of protein. Biotechnol Prog. 2005;21:516–23

[25]. **Kyte, J. and Doolittle, R.F. (1982)** A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157, 105-132. [PubMed: 7108955].

[26]. **Wei BQ.** "Testing a flexible-receptor docking algorithm in a model binding site". Journal of Molecular Biology 337.5 (2004): 1161-1182.

[27]. **Jin, H. R.; Zhao, J.; Zhang, Z.; Liao, Y.; Wang, C. Z.; Huang, W. H.; Li, S. P.; He, T. C.; Yuan, C. S.; Du, W. (2012).** "The antitumor natural compound falcarindiol promotes cancer cell death by inducing endoplasmic reticulum stress". Cell Death and Disease. 3 (8): e376. doi:10.1038/cddis.2012.122. PMC 3434669. PMID 22914324.

[28]. **ARNOTT, J. A. & PLANEY, S. L. (2012).** The influence of lipophilicity in drug discovery and design. Expert Opinion on Drug Discovery 7, 863–875.

[29]. **ARTURSSON, P. & KARLSSON, J. (1991).** Correlation between oral-drug absorption in humans and apparent drug permeability coefficients in human intestinal epithelial (Caco-2) cells. Biochemical and Biophysical Research Communications 175, 880–885.

[30]. **Lipinski CA (2004)** Lead- and drug-like compounds: the rule of-five revolution. Drug Discovery Today: Technologies 1(4): 337-341.

[31]. **Ikpeazu OV, Otuokere IE, Igwe KK (2017)** In Silico Structure-Activity Relationship and Virtual Screening of Monosubstituted Doxycycline with Pseudomonas Aeruginosa Lipase. J Anal Pharm Res 5(3): 00139. DOI: 10.15406/japlr.2017.05.00139.

[32]. **CLARK, D. E. (2003).** In silico prediction of blood-brain barrier permeation. Drug Discovery Today 8, 927–933.

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