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Anti-Fungal Activity of a Secondary Metabolite of *Lawsonia alba* and its Derivatives – An *in-silico* Study

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Abstract:

Introduction:
Fungal infections of skin, hair, and nails, are common and the incidence rate of these diseases were continuously increasing around 20–25% of the world's population. The clinically important fungal infections are characterized by primary or opportunistic. Cutaneous fungal infections and the emerging multidrug resistance are driving interest in fighting these microorganisms with natural products. Endoproteases are responsible for the cleavage of the various proteins and produce C or N compounds that enable the activity of exoproteases, which is cause for availability of aminoacids like nutrient material for the survival of fungal organism. The phytochemical analysis of the aqueous and ethanol extracts of the leaves of *Lawsonia inermis* revealed the availability of myriad of secondary metabolites. This lawsone is having the ability to inhibit the invasion of fungal organism probably by inhibiting the M36 metalloendopeptidase known as Fungalysin. By this inhibition it can arrest the purulence of fungal diseases.

Methods: The 3D structure of Fungalysin was not available in the pubchem database. Homology model was prepared with software and validated. The physicochemical parameters were obtained for the secondary metabolite, Lawsone and the derivatives and these were docked. The derivatives were generated by SWISS ADME online tool. The files were interconverted with OPENBABEL from .mol to .sdf and .pdb files.

Results: The protein and ligand were docked and posed with Pymol and the binding affinity values were obtained by Autodock Vina and were tabulated. The Van der Waals force between the protein and ligands were generated by iGEMDOCK and tabulated.

Conclusion: The Fungalysin and Lawsone have a good binding affinity and thereby inhibits the targeted protein thereby inhibits the invasion of fungal organism.

Key words: Fungalysin, Metalloprotease, *Lawsonia alba*, Lawsone, iGEMDOCK, Autodock Vina, OPENBABEL.

INTRODUCTION:

Fungi are eukaryotic microorganisms that occur ubiquitously in nature. The basic morphological element of filamentous fungi is the hyphae and a web of intertwined hyphae. Fungal infections represent a critical problem for morbidity and mortality worldwide[1]. Infections of skin and mucosal surfaces, constitute a serious problem, in tropical and subtropical developing countries[2]. The fungal infections of skin, hair, and nails, are common and the incidence rate is increasing around 20–25% of the world's population[3]. Dermatophytes are keratophilic fungi with genera Trichophyton, Microsporum, and Epidermophyton which causes Cutaneous mycotic infections and they are capable of surviving even in the absence of keratin but in a superficial infection they are capable of generating and releasing a special type of enzymes that allow to invade the host's keratinized tissues[4-8]. Fungal infections and the emerging multidrug resistance are driving interest for natural products. Medical scientists have realized the pathology and progression of illness like fungal infections are complicated that the therapeutic effect of the drugs may be hampered by various adverse events or resistance in clinic[9-11].

Dermatophytes are true pathogenic fungi producing Metalloprotease during infection to mammals is seen to have a role in dermatophytic virulence[12]. The dermatophytes are in generally have proteolytic activity observed in *in-vitro* studies[13]. In many studies the isolation and characterization of one or two proteases from the species of Dermatophytes[14-16] are described as keratinases. Different research papers have shown that these enzymes play an important role in the provision of nutrients[17], in host tissue invasion[18] and in the control of host defense mechanisms[19-20]. Endoproteases are responsible for the cleavage of the various proteins and produce a high number of Carbon and/or Nitrogen terminus compounds that enable the activity of exoproteases, which is cause for availability of aminoacids like nutrient material for the survival of fungal organism[21].

The Ayurvedic Pharmacopoeia of India indicated the use of the *Lawsonia alba* leaves in dysuria, jaundice, bleeding disorders, ulcers, and other obstinate skin diseases. Lawsone is the well known secondary metabolite of *Lawsonia alba* and the phytochemical analysis of the aqueous and ethanol extracts of the leaves of *Lawsonia inermis* revealed the availability of alkaloids, carbohydrates, resins, saponins, sterols and tannins in different composition in the aqueous extract, fractions of ethanol extract and fractionation residue of the leaves in Nigeria[22]. Gas Chromatography – Mass Spectrometry screening observed that the essential oil of the leaves of *Lawsonia inermis* (henna) had thirty-six components which constituted 80.4% of the oil. The major components were ethyl hexadecanoate (24.4%), (E)-methyl cinnamate (11.4%), isocaryophyllene (8.1%), (E)-beta-ionone (5.8%) and methyl linolenate (4.1%)[23].

Fungalysin (M36 family) is a Metalloprotease which is exclusively present in Fungi which was used as a drug target to inhibit the invasion of fungi to host internal environment. The software is helpful in analyzing the protein, the target for drug action with possible predicted active site, identifying lead molecule, check for druglikeliness, dock the proteins with ligand, hierarched based on binding affinity.

MATERIALS AND METHODS:

Preparation of Protein:

Fungalysin is a metallopeptidase (MMP3) plays a vital role in the synthesis of fungal cell wall. The 3D structure of this protein is not available in Pubchem data base. The homology modeling of this macromolecule was generated.

Homology Modeling:

Homology modeling has been developed with the help of software "Easy Modellar". The query sequence and the template were retrieved from NCBI, the query sequence was aligned with the template sequence and the model was generated. The generated 3D structure of the macromolecule was validated by Ramachandran Plot.

Preparation of Ligand:

Lawsone is a small molecule present as a secondary metabolite in the plant *Lawsonia alba*. The SMILES of the ligand was directly obtained from pubchem database which is a free database available for compounds for virtual screening. From the pubchem database the structure was downloaded in .sdf file format. The .sdf file was converted into .pdb file with software OPENBABEL. SWISS ADME online tool was used to get the 112 derivatives (Structure Activity Relative molecules) of the Lawsone. The same SWISS ADME software was used to generate the physicochemical, medicinal, and druglikeliness properties of the secondary metabolites of Lawsone and the derivatives. The best suited 10 derivatives were selected from the 112 Structure Activity Relative molecules based on the binding affinity. Rough docking was done with iGEMDOCK2.0 software with a population size of 150 and 70 generations was set as default. Lipinski's rule[24, 25] also called as the Rule of five (RO5) is a rule of thumb to evaluate the druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that may likely active per orally in human beings.

Protein –Ligand Docking:

The protein-ligand docking was performed by Autodock Vina, an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and ligands and were presented hierarchically based on binding affinities.

RESULTS:

Protein-ligand preparation:

The homology model of target protein, Fungalysin was docked with the small molecule called Lawsone and also with the 112 derivatives or structure activity relative compounds. The homology model for drug target was validated with the Ramachandran plot was shown in Figure 01. The 10 derivatives were selected based on the binding affinity and the physicochemical, medicinal, and druglikeliness properties. The docked poses of the Fungalysin with Lawsone and the selected derivatives were shown in Figure 02. The energy values, Van der Waals force, H-bond were derived by rough docking with a software iGEMDOCK for Fungalysin with Lawsone and the selected derivatives were presented in Table 01. The binding affinity of the docked protein and ligand was obtained on accurate docking with Autodock Vina for Fungalysin with Lawsone and the selected derivatives were presented in Table 02. The chemical nature like chemical formula, structure, SMILES (Simplified Molecular Input Line Entry System), and IUPAC (International Union of Pure and Applied Chemistry) for the Lawsone and the derivatives were presented in Table 03. The physicochemical properties like molecular weight heavy atoms, Fraction CSP3, rotatable bonds, H-bond acceptors, H-bond donors, Molecular refractivity, TPSA(Topological Polar Surface Area) for the Lawsone and the derivatives was presented in Table 04. The Lipophilicity and Hydrophilicity for the Lawsone and the derivatives were shown in Table 05. The pharmacokinetic properties for the Lawsone and the derivatives were presented in Table 06.

In Table 1 Lawsone shows energy values as -78.84 and Van der Waals force -65.01 between protein and ligand. The structure activity relative (SAR1) molecule as derivative 1 was showing the nearest to the secondary metabolite as energy values -71.1438 and Van der Waals force -54.1827.

In Table no 2 shows that the binding affinity between protein, Fungalysin and the ligand Lawsone was -7.9 and the derivative 43, Structure Activity Relative (SAR 4) molecule was -6.4 which is the nearest to the secondary metabolite.

Table 3 shows the general properties like molecular formula, chemical structure, SMILES, and IUPAC name of a secondary metabolite of the secondary metabolite of *Lawsonia alba*, Lawsone and the derivatives.

In Table 4 showing molecular weight, no of atoms, fraction CSP3, no of rotatable bonds, molar refractivity, and Topological Polar Surface Area, where it shows that the molecular weight is more than less than 200, no of atoms are in the permissible range is less than 20, molar refractivity is less than 60, polar surface area is also more than 30 angstroms squared in Lawsone and the derivatives.

The Table 5 is showing the log p Octanol - Water partition co-efficient values of the Lawsone and the derivatives are in the range of permissible -0.4 to +5.6 range that implies the lawsone and the derivatives are a good lipophilic compounds. The consensus log $P_{o/w}$ means average of all five predictions is also in the permissible range. Hydrophilicity of the lawsone and derivatives show soluble and very soluble nature.

The Table 6 is showing the pharmacokinetic property of Lawsone and the derivatives, implies an excellent oral bioavailability, crosses blood brain barrier except derivative 02(SAR 2), inhibits cytochrome P 450 1A2 isoform except Lawsone and derivatives 1(SAR 1), 29(SAR 3), 43(SAR 4), 57(SAR 5),71(SAR 6), 100(SAR 9).

Ramachandran plot:

The Ramachandran plot is helpful to visualize the dihedral angles ψ (phi) and φ (psi) of a macromolecule or protein backbone[26] was discovered by G. N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan[27]. The Torsion angles for loop regions in a given protein do not often occupy particular regions in the plot unlike secondary structure elements such as α-helices or β-sheets, but they may occupy any regions that are sterically permitted. The protein, Fungalysin structure was validated by Ramachandran plot it was inferred that the modeled protein contains 96.9% of amino acid residues in the favoured region, 2.3% in allowed region, and 0.8% in amino acid residues in disallowed region.

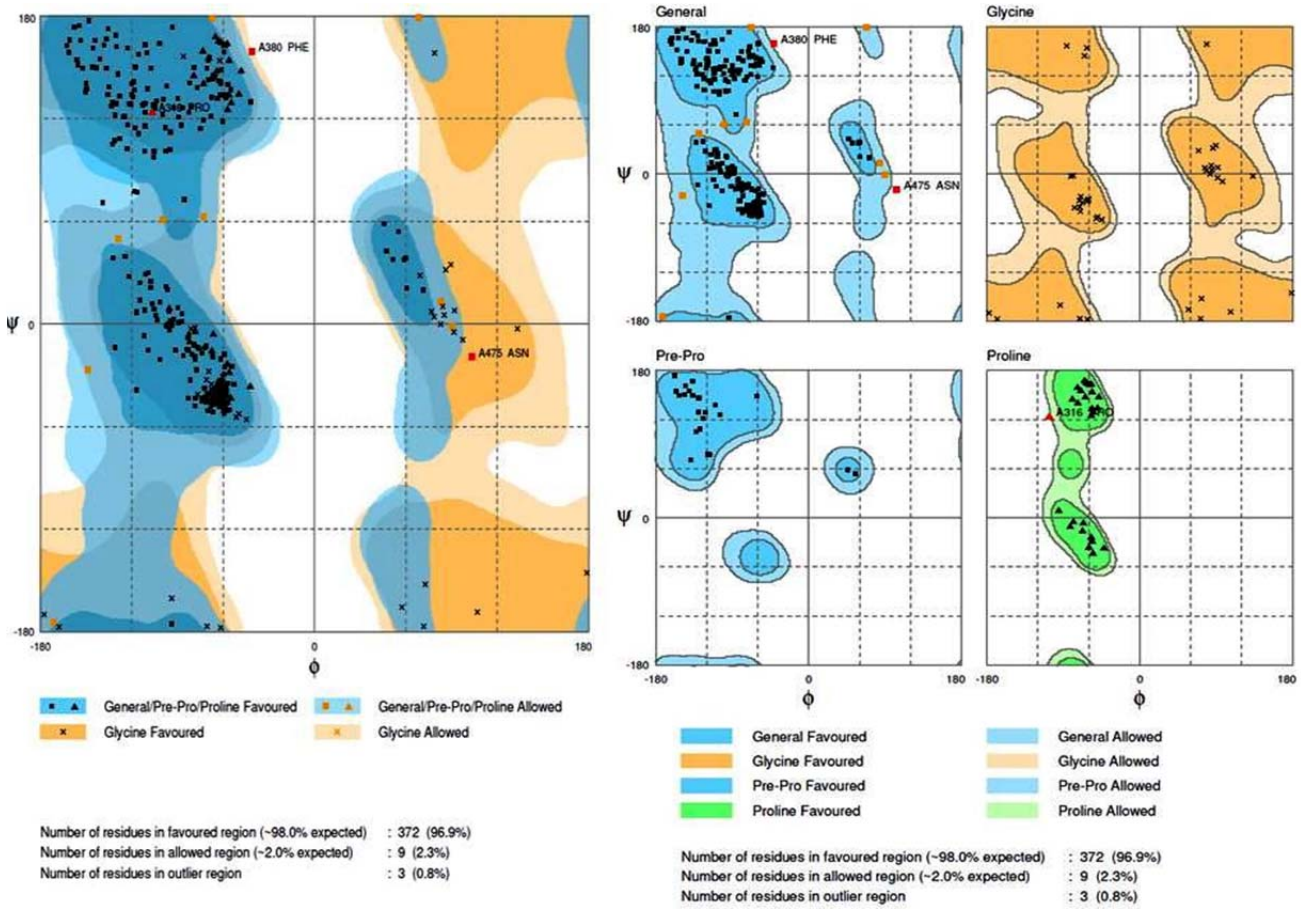


Figure 1: **Ramachandran plot shows the number of aminoacids that are favoured, allowed and disallowed in model protein FUNGALYSIN**.

Table 1: **The results of rough docking for Fungalysin with Lawsone and the derivatives was performed with iGEMDOCK**.

S no	Protein with Ligand	Total Energy	VDW	H Bond	Elec	Aver Con Pair
1	FUNGALYSIN – LAWSONE	-78.8417	-65.0134	-13.8283	0	30
2	FUNGALYSIN – LAWSONE DERIVATIVE NO 01(SAR 1)	-71.1438	-54.1827	-16.9611	0	23.7143
3	FUNGALYSIN – LAWSONE DERIVATIVE NO 02(SAR 2)	-60.9249	-50.3759	-10.549	0	22.9286
4	FUNGALYSIN – LAWSONE DERIVATIVE NO 29(SAR 3)	-67.3149	-49.0742	-18.2407	0	32.7143
5	FUNGALYSIN – LAWSONE DERIVATIVE NO 43(SAR 4)	-63.0279	-49.7233	-13.3046	0	24.1429
6	FUNGALYSIN – LAWSONE DERIVATIVE NO 57(SAR 5)	-66.2235	-52.5406	-13.6829	0	23.9286
7	FUNGALYSIN – LAWSONE DERIVATIVE NO 71 (SAR 6)	-68.6861	-53.3024	-15.3837	0	24.3571
8	FUNGALYSIN – LAWSONE DERIVATIVE NO 87 (SAR 7)	-65.4585	-51.5868	-13.8717	0	28.4615
9	FUNGALYSIN – LAWSONE DERIVATIVE NO 99(SAR 8)	-62.7747	-50.7747	-12	0	26.3333
10	FUNGALYSIN – LAWSONE DERIVATIVE NO 100(SAR 9)	-67.2347	-51.7419	-15.4927	0	24.3846
11	FUNGALYSIN – LAWSONE DERIVATIVE NO 101(SAR 10)	-68.6881	-53.1881	-15.5	0	23.3846

Table 2: **The results showing the binding affinity of Fungalysin with Lawsone and the derivatives**.

S no	Name of the protein and ligand	Binding Affinity	RMSD/UB (Root Mean Square Deviation/Upper Bound)	RMSD/LB (Root Mean Square Deviation/Lower Bound)
1	FUNGALYSIN – LAWSONE	-7.9	0	0
2	FUNGALYSIN – LAWSONE DERIVATIVE NO 01(SAR 1)	-6	0	0
3	FUNGALYSIN – LAWSONE DERIVATIVE NO 02(SAR 2)	-6.2	0	0
4	FUNGALYSIN – LAWSONE DERIVATIVE NO 29(SAR 3)	-6.1	0	0
5	FUNGALYSIN – LAWSONE DERIVATIVE NO 43(SAR 4)	-6.4	0	0
6	FUNGALYSIN – LAWSONE DERIVATIVE NO 57(SAR 5)	-6	0	0
7	FUNGALYSIN – LAWSONE DERIVATIVE NO 71 (SAR 6)	-5.9	0	0
8	FUNGALYSIN – LAWSONE DERIVATIVE NO 87 (SAR 7)	-5.9	0	0
9	FUNGALYSIN – LAWSONE DERIVATIVE NO 99(SAR 8)	-6.1	0	0
10	FUNGALYSIN – LAWSONE DERIVATIVE NO 100(SAR 9)	-6	0	0
11	FUNGALYSIN – LAWSONE DERIVATIVE NO 101(SAR 10)	-6.1	0	0

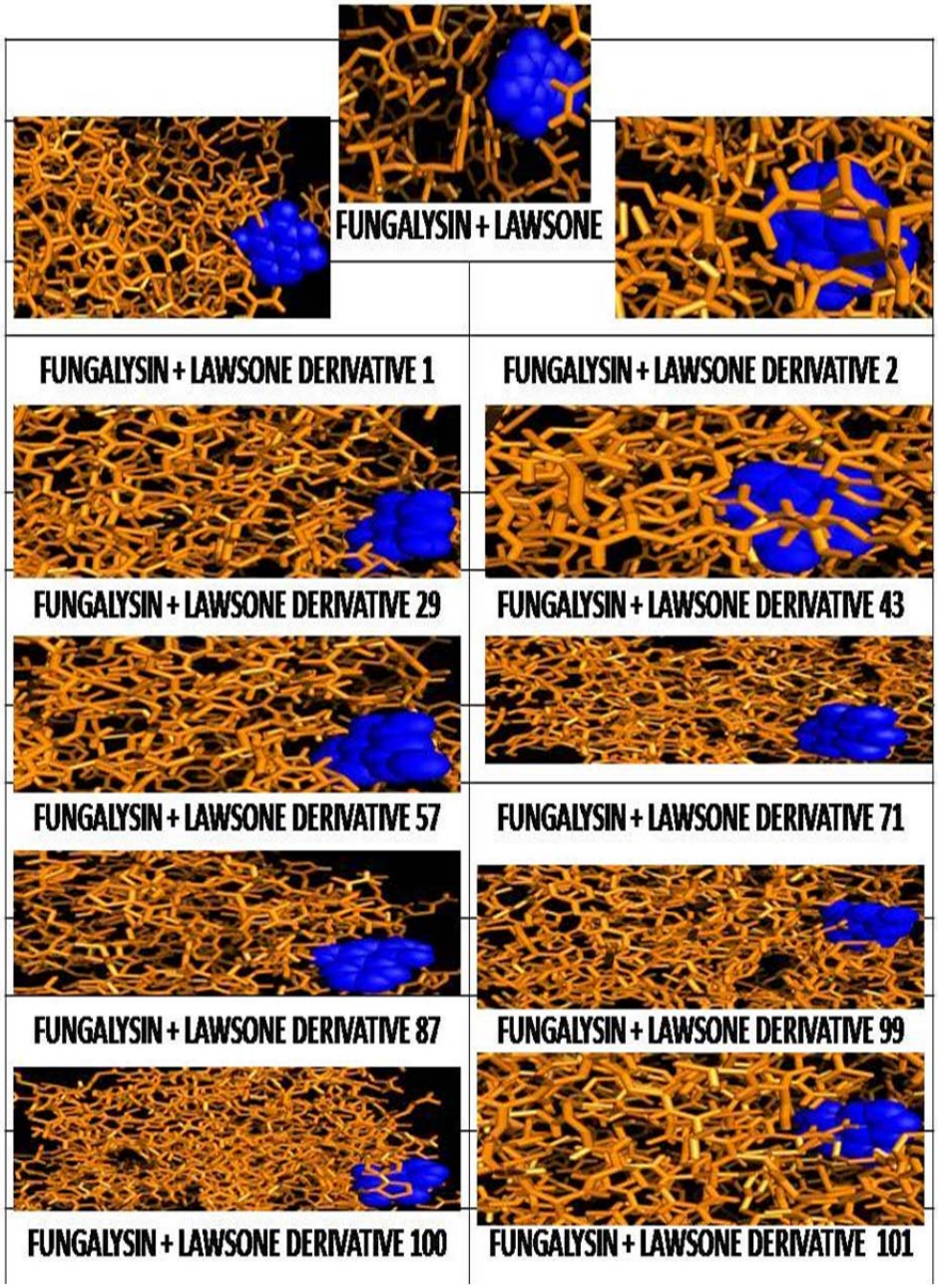


Figure 2: **The picture showing the docking poses of Lawsone and the derivatives**.

Table 3: The General properties of Lawsone and the derivatives.

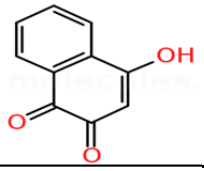
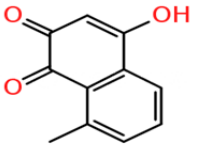
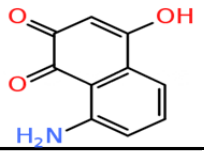
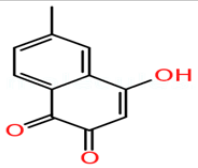
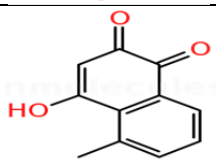
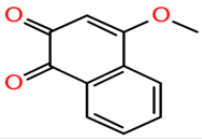
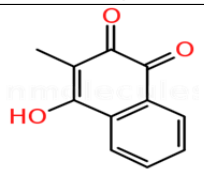
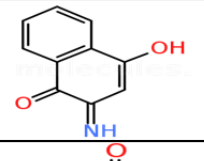
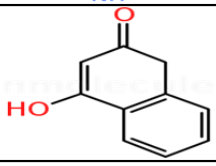
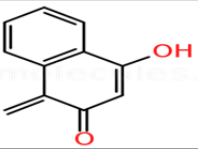
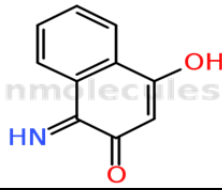
Name the of Ligand	Chemical Formula	Chemical Structure	SMILES	IUPAC
LAWSONE	C10H6O3		O=C1C=C(O)c2c(C1=O)cccc2	4-hydroxynaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 01(SAR 1)	C11H8O3		OC1=CC(=O)C(=O)c2c1cccc2C	4-hydroxy-8-methyl-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 02(SAR 2)	C10H7NO3		OC1=CC(=O)C(=O)c2c1cccc2N	8-amino-4-hydroxy-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 29(SAR 3)	C11H8O3		Cc1ccc2c(c1)C(=CC(=O)C2=O)O	4-hydroxy-6-methyl-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 43(SAR 4)	C11H8O3		O=C1C=C(O)c2c(C1=O)cccc2C	4-hydroxy-5-methyl-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 57(SAR 5)	C11H8O3		COC1=CC(=O)C(=O)c2c1cccc2	4-methoxy-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 71 (SAR 6)	C11H8O3		O=C1C(=O)C(=C(c2c1cccc2)O)C	4-hydroxy-3-methyl-1,2-dihydronaphthalene-1,2-dione
LAWSONE DERIVATIVE NO 87 (SAR 7)	C10H7NO2		N=C1C=C(O)c2c(C1=O)cccc2	4-hydroxy-2-imino-1,2-dihydronaphthalen-1-one
LAWSONE DERIVATIVE NO 99(SAR 8)	C10H7O2		O=C1C=C(O)c2c(C1)cccc2	4-hydroxy-1,2-dihydronaphthalen-2-one
LAWSONE DERIVATIVE NO 100(SAR 9)	C11H8O2		O=C1C=C(O)c2c(C1=C)cccc2	4-hydroxy-1-methylidene-1,2-dihydronaphthalen-2-one
LAWSONE DERIVATIVE NO 101(SAR 10)	C10H7NO2		O=C1C=C(O)c2c(C1=N)cccc2	4-hydroxy-1-imino-1,2-dihydronaphthalen-2-one

Table 4: The Physicochemical Properties of Lawsone and the derivatives.

Name of the ligand	Molecular Weight (g/mol)	Num. heavy atoms	Num. arom. heavy atoms	Fraction CSP3	Num. rotatable bonds	Num. H-bond acceptors	Num. H-bond donors	Molar Refractivity	Topological Polar Surface Area (TPSA) $^{o}A^{2}$
LAWSONE	174.15	13	6	0.00	0	3	1	46.39	54.37
LAWSONE DERIVATIVE NO 01(SAR 1)	188.18	14	6	0.09	0	3	1	51.35	54.37
LAWSONE DERIVATIVE NO 02(SAR 2)	189.17	14	6	0	0	3	2	50.79	80.39
LAWSONE DERIVATIVE NO 29(SAR 3)	188.18	14	6	0.09	0	3	1	51.35	54.37
LAWSONE DERIVATIVE NO 43(SAR 4)	188.18	14	6	0.09	0	3	1	51.35	54.37
LAWSONE DERIVATIVE NO 57(SAR 5)	188.18	14	6	0.09	1	3	0	50.71	43.37
LAWSONE DERIVATIVE NO 71 (SAR 6)	188.18	14	6	0.09	0	3	1	51.19	54.37
LAWSONE DERIVATIVE NO 87 (SAR 7)	173.17	13	6	0	0	3	2	49.78	61.15
LAWSONE DERIVATIVE NO 99(SAR 8)	159.16	12	6	0	0	2	1	47.22	37.3
LAWSONE DERIVATIVE NO 100(SAR 9)	172.18	13	6	0	0	2	1	51.09	37.3
LAWSONE DERIVATIVE NO 101(SAR 10)	173.17	13	6	0	0	3	2	49.55	61.15

Table 5: The Lipophilicity and hydrophilicity of Lawsone and the derivatives.

Name of the Ligand	Lipophilicity	Hydrophilicity		
	Consensus Log $P_{o/w}$	Log S (ESOL)	Solubility (mg/ml)	Class
LAWSONE	0.96	-1.80	2.78e-00	Very soluble
LAWSONE DERIVATIVE NO 01(SAR 1)	1.29	-2.09	1.52E+00	Soluble
LAWSONE DERIVATIVE NO 02(SAR 2)	0.55	-1.78	3.11E+00	Very soluble
LAWSONE DERIVATIVE NO 29(SAR 3)	1.29	-2.09	1.52E+00	Soluble
LAWSONE DERIVATIVE NO 43(SAR 4)	1.29	-2.09	1.52E+00	Soluble
LAWSONE DERIVATIVE NO 57(SAR 5)	1.35	-2	1.88E+00	Soluble
LAWSONE DERIVATIVE NO 71 (SAR 6)	1.35	-2.09	1.52E+00	Soluble
LAWSONE DERIVATIVE NO 87 (SAR 7)	1.16	-1.85	2.46E+00	Very soluble
LAWSONE DERIVATIVE NO 99(SAR 8)	1.39	-1.97	1.70E+00	Very soluble
LAWSONE DERIVATIVE NO 100(SAR 9)	1.85	-2.42	6.53E-01	Soluble
LAWSONE DERIVATIVE NO 101(SAR 10)	1.1	-1.85	2.46E+00	Very soluble

Table 6: The Pharmacokinetics properties of a secondary metabolite of *Lawsonia alba*, Lawsone and the derivatives.

Name of Ligand	GI absorption	BBB Permeability	P-gp Substrate	CYP 1A2 Inhibitor	CYP2C19 Inhibitor	CYP2C9 Inhibitor	CYP2D6 Inhibitor	CYP3A4 Inhibitor	Log K_p (skin permeation) cm/s
LAWSONE	HIGH	YES	NO	NO	NO	NO	NO	NO	-6.76
LAWSONE DERIVATIVE NO 01(SAR 1)	High	Yes	No	Yes	No	No	No	No	-6.58
LAWSONE DERIVATIVE NO 02(SAR 2)	High	No	No	No	No	No	No	No	-6.94
LAWSONE DERIVATIVE NO 29(SAR 3)	High	Yes	No	Yes	No	No	No	No	-6.58
LAWSONE DERIVATIVE NO 43(SAR 4)	High	Yes	No	Yes	No	No	No	No	-6.58
LAWSONE DERIVATIVE NO 57(SAR 5)	High	Yes	No	Yes	No	No	No	No	-6.61
LAWSONE DERIVATIVE NO 71 (SAR 6)	High	Yes	No	Yes	No	No	No	No	-6.58
LAWSONE DERIVATIVE NO 87 (SAR 7)	High	Yes	No	No	No	No	No	No	-6.69
LAWSONE DERIVATIVE NO 99(SAR 8)	High	Yes	No	No	No	No	No	No	-6.4
LAWSONE DERIVATIVE NO 100(SAR 9)	High	Yes	No	Yes	No	No	No	No	-6.03
LAWSONE DERIVATIVE NO 101(SAR 10)	High	Yes	No	No	No	No	No	No	-6.69

DISCUSSION:

The fungal diseases are a major threat in the world scenario in history. Most of the fungal infections in mammals are superficial and relatively innocuous, but some can cause devastating diseases like invasive aspergillosis and systemic candidiasis. The clinically important fungal infections are characterized by primary or opportunistic. Primary fungal infections may cause infection in healthy population even those are not exposed to endemic fungi, while opportunistic infections are mostly causes to immunosuppresed individuals[28]. The development of antifungal drug resistance and the adverse events are the major contribution for the morbidity and mortality. The medical devices and medical services in healthcare lead to increase in number of susceptible individuals. The emergence and re-emergence of infections caused by fungal organisms like candidiasis, aspergillosis, cryptococcosis, mucormycosis and pneumocystosis are the commonest. But Candidemia is the leading cause of blood-stream infection with mortality rate more than 30%[29] whereas Aspergillus can affect more than 45% of susceptible host[30]. Zygomycosis is common among diabetics all around the globe including India[31]. Mortality associated with invasive fungal infections among patients hospitalized in ICUs has reached to 67%[30]. Fungi are evolved and adapted in a variety of environmental and ecological niches. The safe and selective target to the fungi and safe for host is well required to avoid any harm to the recipient. The treasure source for safe alternatives is a medicinal plants which has number of events showed as evidence. Medicinal plants used in skin diseases including mycotic infection is an old practice[32]. The secondary metabolites in the medicinal plants and their antimicrobial activity against infective pathogens has focused by several workers[33]. The currently available drugs for fungal diseases are Griseofulvin, Terbinafine, Amphotericin B, Nystatin, Flucytosine, Clotrimazole, Miconazole, Ketoconazole, Fluconazole, Benzoic Acid, Salicylic Acid, Tolnaftate[34].

The *In-silico* method of drug discovery is aiding to discover new drug targets and newer ligands using software to identify the lead compounds and also can reduce pre-clinical study period. Fungalysin selected from literature as a drug target whose 3D structure was not available in National Center for Biotechnology Information. The homology model was generated with EASY MODELLAR and validated with Prochek of Ramachandran plot. Lawsone, a secondary metabolite was selected as ligand. The protein and ligand interaction was docked with software's, iGEMDOCK and Autodock Vina[35], the results were collected on the basis of energy values, Van der Waals forces, binding affinities between the protein and ligand. The structure activity relative molecules were retrieved with soft ware SWISS ADME[36, 37] online tool. In a study by Emin Zumrutdal and Mehmet Ozaslam, Lawsonia inermis was shown high percentage of fungal growth inhibition which is around 76.47% - 87.77%. According to Khan and Nasreen, the proteins and proteinacious compounds of the compounds like Lawsone are effective against plant pathogens[38]. The Lawone and the derivatives are obeys the Lipinski's rule of 5 and other filters for a new drug molecule, and the bioavailability score is 0.56.

CONCLUSION:

In this study the secondary metabolite Lawsone and the derivatives are showing inhibitory action against the model protein Fungalysin, a Metalloprotease(M36). It was suggested that the protein ligand interaction for a new drug target and a secondary metabolite of *Lawsonia alba* or with the selected derivatives have an interesting new drug target and new ligand.

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