Virtual Screening and *in silico* Determination of Antifungal Property of Medicinal Plant Compounds Using QSAR Studies

Sharanya Manoharan[1]*, Suganya Jeyabasker[1], Poornima Vasudevan[1], Radha Mahendran[2]

[1]Assistant Professor, [2]Professor
Department of Bioinformatics, Vels University, Chennai-600 117, Tamil Nadu, India.

Abstract **:** The era of identifying antibiotics to treat bacterial endemic conditions is the beginning for the emergence of fungal infections as a common health threat among the population. Infections caused by fungi are superficial, cutaneous and sub-cutaneous infections of the skin, rhinosinusitis, mycetism, mycotoxicosis, otomycosis and occulomycosis. Cutaneous fungal infections are often divided into 'superficial' and 'deep' forms. The existing classes of antifungal agents are polyenes, azoles, pyrimidines, allylamines, candins and the drug griseofulvin, each targeting the fungal cell with their unique mode of action. Long course administration of drugs has its own effect on the decrease in fungal sensitivity to the antifungal agents. Natural plant products, in general are recognized as healthier than manufactured medicines and also medicinal plants are the reservoir of small molecules, hence tracking the activity of the plants and identifying the compounds with specific activity may help in identifying the candidate inhibitors. QSAR study was employed to determine the antifungal biological activity of the plant compounds which obeyed the Lipinski's rule of five, determined through QED and molinspiration. Among 300 compounds, only 52 showed drug-likeness. Further on QSAR analysis, the compounds obtusin, thymohydroquinone and aloe emodin exhibited antifungal property when determined with the descriptors like molecular volume, molecular weight, polar surface area, number of rotatable bonds, number of aromatic rings, donor and acceptor. These three compounds would be explored for future perspectives like docking studies and pharmacophore mapping.

Keywords: Fungal skin infections, Antifungal Plants, QED, Molinspiration, Drug-likeness, QSAR.

I. INTRODUCTION

Fungal infections (also called mycoses) is known worldwide as fungal pathogens causing infections in enormous plants and animals and are recently identified as responsible for species extinctions, food spoil and ecosystem disturbances. So far, U.S. centers for disease control and prevention (CDC) is the only public health agency to conduct survey on mycological infections. Even more World Health Organization (WHO) itself has no program to monitor the fungal infection (Brown et al., 2012). Fungal infections are generally classified into superficial and invasive, where in the former the skin and nails are commonly infected by the causative organism called dermatophytes and the latter includes candidiasis, aspergillosis, and etc. that are responsible for high mortality rates (Brown et al., 2012a). The fungal genera, *Cryptococcus*, *Candida*, *Aspergillus* and *Pneumocystis* are reported as causative for 90% of fungal-related deaths. Fungal infections are highly concerned among the patients with poor immune system i.e. HIV/AIDS, organ transplantation, etc. (Brown et al., 2012). The existing common antifungal agents are polyenes, pyrimidines, azoles, allylamines, morpholines and echinocandins, despite exhibit their unique inhibitory mechanism, they fails to serve its responsibility to the fullest. This is because, the fungi are being an eukaryote shares features in common with the human beings, which perhaps on treating the disease for long time leads to side effects in the host (Sanglard, 2002). Moreover, the fungi develops resistance towards the antifungal agents by attaining specific mechanism on prolong exposure (Sanglard, 2002). Though the mortality rate of fungal infections are high as tuberculosis and malaria, they are understudied and underdiagnosed as well as the good epidemiological data are lacking. Therefore, general awareness among the population is extremely important and robust, rapid, simple and cheap diagnostics are to be developed (Brown et al., 2012b).

From ancient times, India depends mostly on the plants for food, health and other basic needs. In the present era, more number of secondary metabolites were isolated, identified and scientifically evaluated for its significant medicinal properties. Also the 80% population of developing countries depends on plants for curing different ailments due to their cost effective and safety (Verma, 2016). Bioinformatics is emerging techniques combining biology and computer science. In the present study, quantitative structure activity relationship (QSAR) was used relate the biological activity of the plant secondary metabolites with their physical as well as chemical properties. The QSAR parameters could be utilized for measuring specific property of the parent group and its group.

II. MATERIALS AND METHODS

2.1 Retrieval of Compounds

The present study focused on the plant compounds from the medicinal plants *Acorus calamus* (Paithankar et al., 2011), *Ageratum conyzoides* (Kamboj and Saluja, 2008), *Aloe vera* (Maan et al., 2018), *Amaranthus spiransus* (Ulbricht et al., 2009), *Anacyclus pyrethrium* (Usmani et al., 2016), *Artemisia indica(*Rashid et al.,2013*), Benincasa hispida (*Sharma et al.,2014), *Cassia alata(*Timothy et al.,2012*), Cassia fistula (*Bhalodla et al., 2012), *Callicarpa arborea (*Umachandur et al .,*)Celantrus peniculatus(*Singh et al.,2010*), Centella asiatica (*Zahara et al.,2014*), Citrullus colocynthis (*Hadizadeh et al.,2009*), Clitoria ternatea(*Kamila et al.,2009*), Colocasia esculenta (*Dutta et al.,2017*), Evolvus alsinoides (*Shukla et al.,2012*), Gynocardia odorata(*Kalita et al.,2018*), Jatropha gassypifolia (*Felix Silva et al.,2014*), Nigella sativa (*Ahamed et al.,2017), *Tacca integrifolia(*Nurul et al.,2009*), Terminalia chebula* (Kamal Rai et al, 2009), *Zingiber zerumbet* (Golam et al.,2011)and *Ziziphus jujube(*Mahajan et al.,2009*).* The compounds reported to be present in the plants through scientific validation were listed to be 300 compounds and the 3D structures were retrieved from the chemical database, PubChem.

2.2 Virtual screening

The compounds based on their descriptors and Lipinki's rule of five can be categorized as either drug-like or non-druglike. The online tools were utilized to predict the descriptors such as polar surface area (PSA), molecular weight, hydrogen bond donor, hydrogen bond acceptor, molecular volume, logP value (octanol-water partition co-efficient), number of rings and number of rotatable bonds. The drug-likeness was determined from quatitative estimation of drug-likeness (an online tool) http://crdd.osdd.net/oscadd/qed/ and the descriptors were calculated from molinspiration (an online tool) https://www.molinspiration.com/cgi-bin/properties. The compounds predicted to drug-like alone subjected from QSAR studies. The 1/logP value was calculated and considered instead of IC50 value for the compounds. For developing QSAR models, the 1/logP value was set as dependent variable and other descriptor values as independent variable. Build QSAR software was used to develop the models as well as for graph determination.

2.3 Graphical Analysis

The graph was plotted using BuildQSAR. Each independent variables were considered along the X-axis and the dependent variables on Y-axis. From the diagonal line obtained, the molecules located near the regression line would represent good correlation i.e. biological activity significantly related to its descriptor value.

2.4. Docking Studies

The molecules identified as significant model in QSAR study was further selected for docking studies. Docking was performed using ArgusLab software. The protein chitinase A (PDB ID: 2XVP) was retrieved from the database PDB (Protein Data Bank) and was prepared by removing the water molecules using ArgusLab and the compounds were loaded in the software. After docking, the results were observed as docking score and the interactions were visualized using Pymol software.

III. RESULTS AND DISCUSSION

DESCRIPTORS GENERATION

About 300 compounds were identified from the plants selected for the study and the drug-likness was predicted using QED. Among 26 compounds only showed drug like property which was further subjected for descriptor generation. The descriptors such as polar surface area (PSA), molecular weight, hydrogen bond donor, hydrogen bond acceptor, molecular volume, logP value (octanol-water partition co-efficient), number of rings and number of rotatable bonds were predicted (Table1). The compounds inhibitory activity (Descriptors) was manually incorporated to the software Build QSAR. And various QSAR models were generated by correlating 1/log P values against any one of the independent variables in MLR analysis.

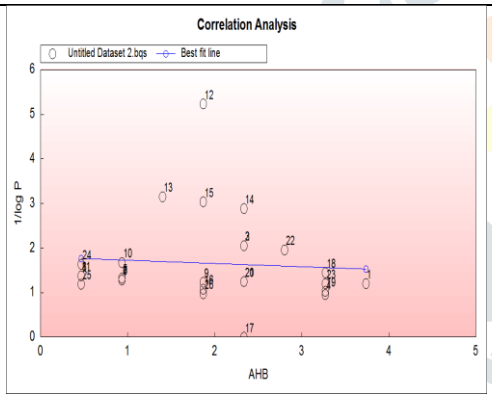
Table 1: Dependent and Independent variables (descriptors) for plant compounds to be used in QSAR models

S. No.	Compound Name	PubchemID	Dependent	Independent						
			1\Log P	MW	DHB	AHB	LogP	NRB	PSA	NOR
1	Ternatin	5459184	0.3132	318.21	2	8	3.192	3	107.59	3
2	Tatarinoids A	46848157	0.5339	221.07	1	5	1.873	1	64.99	1
3	Tatarinoids-B	71551256	0.5339	221.07	1	5	1.873	1	64.99	1
4	Tatarinoids C	46848159	0.2491	366.96	1	7	4.013	2	75.61	2
5	Tatarinowin - A	46848156	0.3454	2.90	1	2	2.895	0	37.3	0
6	Calamendiol	12302239	0.3336	251.90	2	2	2.997	0	40.46	0
7	Iso Calamendiol	12302240	0.3336	251.90	2	2	2.997	0	40.46	0
8	Carvacrol	10364	0.3589	158.57	1	1	2.786	1	20.23	1
9	Quinine	3034034	0.3212	310.79	1	4	3.113	2	45.59	2
10	Thymohydroquinone	95779	0.4345	166.59	2	2	2.301	1	40.46	1
11	Thymol	6989	0.3589	158.57	1	1	2.786	1	20.23	1

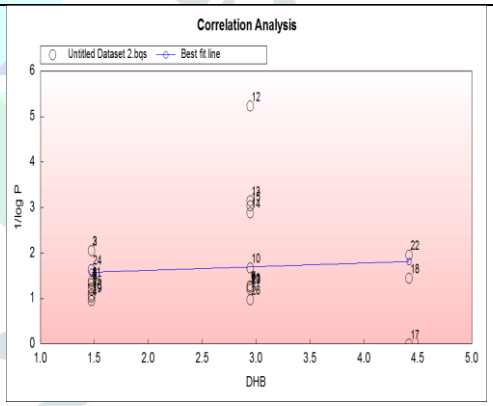
12	Vanillic acid	8468	1.3623	144.61	2	4	0.734	1	45.59	1
13	p-Coumaric acid	637542	0.8196	146.48	2	3	1.220	1	57.53	1
14	Sinapic acid	637775	0.7507	197.57	2	5	1.332	1	75.99	1
15	Ferulic acid	445858	0.7911	172.03	2	4	1.264	1	66.76	1
16	8-O-methylchrysophanol	11300144	0.2810	232.70	1	4	3.558	2	63.6	2
17	Aloe emodin	10207	0.00015	223.43	3	5	2.42	2	94.83	2
18	Aurantio-obtusin	155011	0.3767	274.28	3	7	2.654	2	113.29	2
19	chryso-obtusin	155381	0.2675	309.34	1	7	3.737	2	91.29	2
20	Physcion	10639	0.3253	240.72	2	5	3.074	2	83.83	2
21	Obtusifolin	3083575	0.3253	240.72	2	5	3.074	2	83.83	2
22	Rhein	10168	0.5102	225.61	3	6	1.96	2	111.9	2
23	Obtusin	155380	0.5102	291.81	2	7	3.193	2	102.29	2
24	linalool	6549	0.4260	175.59	1	1	2.347	0	20.23	0
25	α-cadinol	10398656	0.3103	243.65	1	1	3.222	0	20.23	0
26	Nuatigenin	440453	0.2535	427.08	2	4	3.944	0	58.92	0

QSAR MODEL GENERATION

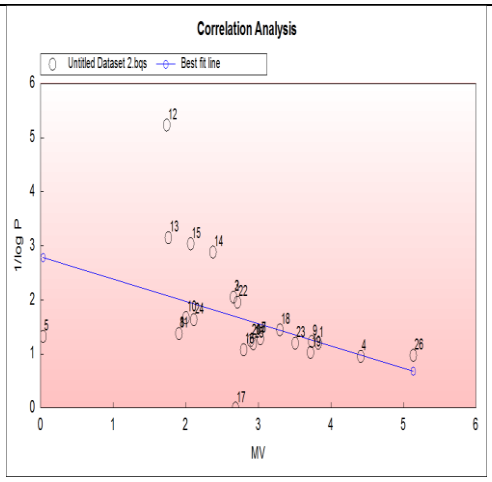
The various models were generated by both independent variables (all descriptors) against the dependent variable 1/logP. Finally the identification of best QSAR model between 1/log P values and structural descriptors could be the major molecular factor are associated with the activity of drug molecule. Further these models were validated using leave-one-out method in MLR analysis. The graphical analysis for the QSAR model has been plotted between the predicted value from the model X-axis and the predicted 1/log P values in Y-axis (Figure 1). The graph depicts that, the compounds aligned on and near the diagonal line displayed the good correlation between predicted 1/log P values and structural descriptors (antifungal activity). Two compounds obtusin and aloe emodin had related all its descriptors with the biological activity. Si et al., (2019) has designed, synthesized a novel pyrazole carboxamide and niacinamide derivatives containing benzimidazole for antifungal activity and 3D QSAR study indicated the significance of structure to correlate its biological function.



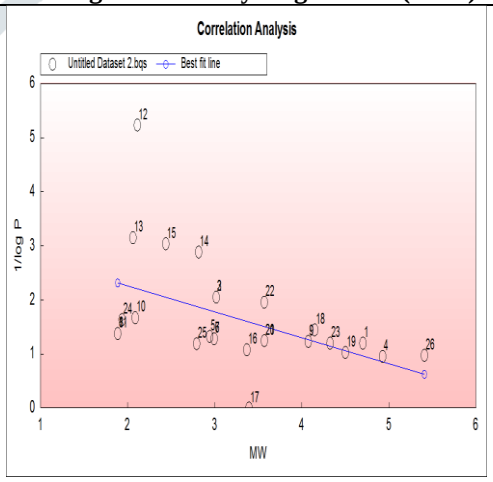
A. 1/log P-Acceptor Hydrogen Bond(AHB)



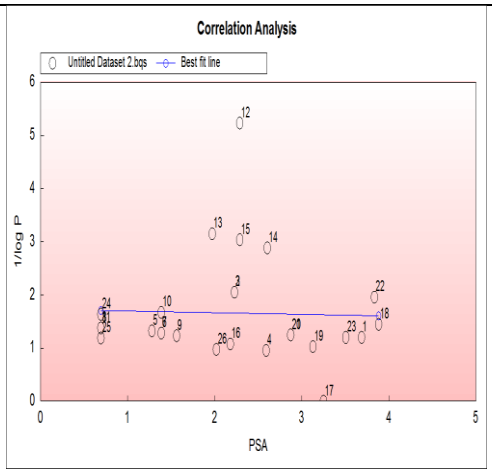
B. 1/log P- Donor Hydrogen Bond(DHB)



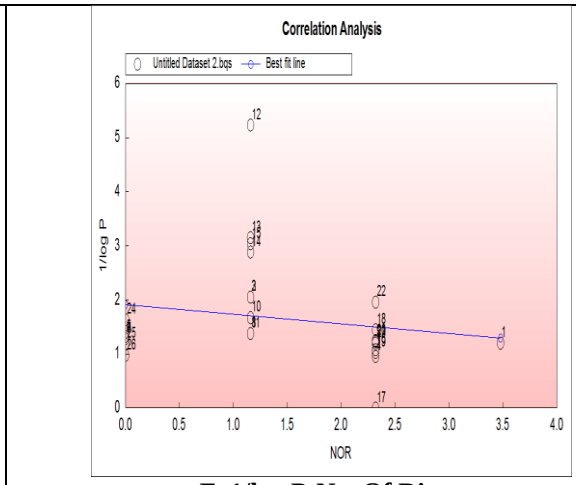
C. 1/log P-Molecular volume(MV)



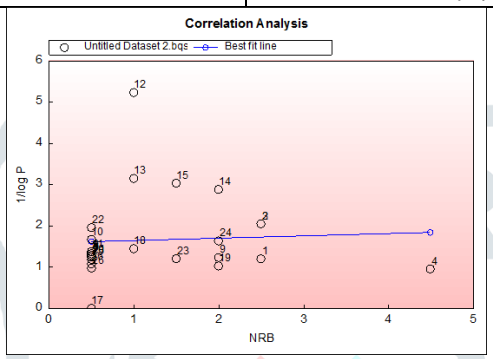
D. 1/log P-Molecular Weight(MW)



E. 1/log P- Polar Surface Area (PSA)



F. 1/log P-No. Of Rings



G. 1/log P-Number Of Rotatable Bond

Figure 1. QSAR models of 1/logP as dependant descriptor against independent descriptors A. Acceptor Hydrogen Bond, B. Donor Hydrogen Bond, C. Molecular volume, D. Molecular Weight, E. Polar Surface Area (PSA), F. Number of rings, G. Number of rotatable bond.

DOCKING STUDIES

Chitinase A is the enzyme involved in the digestion of chitin, a main constituent in fungal cell wall (Figure 2). The active sites were predicted as GLN37**,** PHE60, ALA124**,** TYR125, ASP172**,** GLU174**,** TYR232 andTRP312. The interactions were observed and listed in the table (Table 2) along with binding energy.

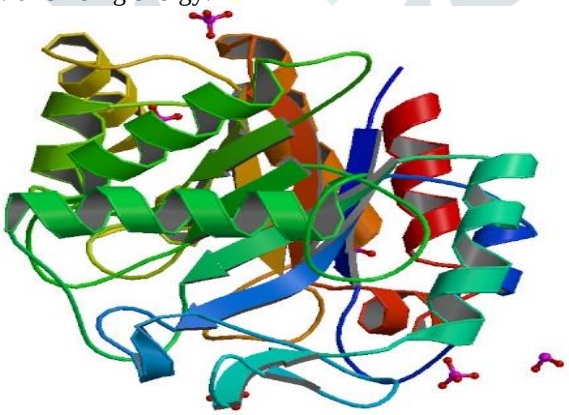
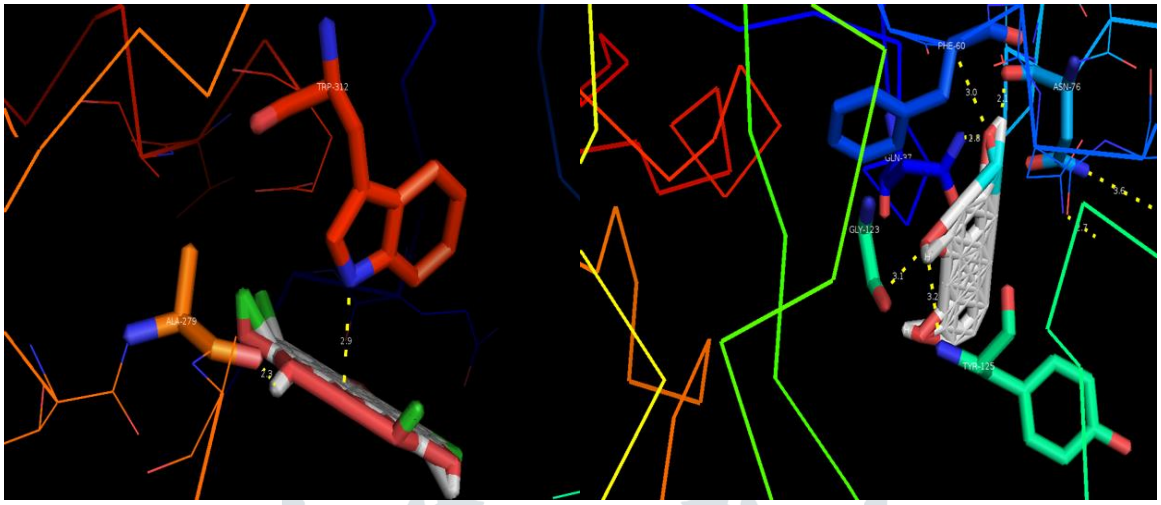


Figure 2: Structure of Chitinase A protein (PDB ID: 2XVP)

The compound obtusin has -9.30 Kcal/mol of binding energy and the interacting residues were Ala279 and Trp312 of bond length 2.3 and 2.9Å, respectively. The compound aloe emodin has -8.49 Kcal/mol of binding energy and formed 5 hydrogen bonds with the following residues: Gln37, Gly123, Phe60, Asn76 and Tyr125 and had respective bond length of 2.8, 1.3, 3.0, 2.1 and 3.2Å. Aloe emodin alone had interacted with the active site residues such as Gln37, Phe60 and Tyr125.

Table 2: Docking results for Chitinase A with plant compounds

S.No.	Compound Name	Binding Energy (Kcal/mol)	Interacting Residues	Bond Length (Å)
1.	Obtusin	-9.30	ALA279 TRP312	2.3 2.9
2.	Aloe Emodin	-8.49	GLN37 GLY123 PHE60 ASN76 TYR125	2.8 1.3 3.0 2.1 3.2



A. OBTUSIN **B. ALOE EMODIN**

Figure 3: Interactions of Chitinase A with the plant compounds

CONCLUSION

The present study revealed that the plant compounds obtusin and aloe emodin had significant correlation between the biological activity and the descriptors included for analysis. Further the docking indicated that aloe emodin to interact with the active site residues predicted for the protein chitinases A. Further studies could be carried out for in vitro experimental analysis of the above compounds as well as the pharmacophore generation.

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