Pharmacophore Based QSAR Modelling of Natural Leads in Antimicrobial Drug Design

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Abstract: To separate and optimize the bioactive fraction of Hemidesmus indicus as an antimicrobial lead using in silico QSAR model development.

Hemidesmus indicus was extracted by soxhalation. The crude extract is fractionated using solvents of different polarity and assessed for its antimicrobial activity. The bioactive fraction is further analysed by GC-MS to analyse the constituents. These moieties were studied for their molecular interaction using CoMFA based QSAR model development.

The bioactive fraction found to have antibacterial and antifungal activities. GC-MS revealed the presence of eicosane, pthalic acid, oleanen 3 -yl acetate and substituted alkanes. Force field analysis followed by simulation revealed that pthalic acid was found to have interaction with the receptors.

Thus the integration of activity guided fractionation with cheminformatics may reveal the putative leads in drug design. Pthalic acid analogues can thus be optimized further by subjecting to preclinical drug studies.

Keywords: COMFA, QSAR, SYBYL, Hemidesmus indicus, antimicrobial drug discovery, infections diseases.

1. INTRODUCTION

Infectious diseases the major cause of death globally next to cancer, diabetes, particularly in infants and the geriatric population [1]. The implication of resistance in the existing anti-microbial review drugs poses a continuous in the quest for the search of new chemical entities. Currently, innovation in the anti-microbial drug discovery experiences a gap due to the emergence of MDR phenotypes and new antibiotic development [2]. Natural products have become an inevitable source of leads in drug discovery. The emergence of integration of combinatorial chemistry with synthetic biology releases surplus chemical scaffolds with improved activities, thus continues to contribute to a great extent with reported diversified pharmacological properties such as antimicrobial, anti-inflammatory, anticancer, etc. Despite its importance, the presence of stereogenic centres, complex scaffolds hinders their lead optimisation process of natural products [3, 4]. Thus in silico modelling involving QSAR (quantitative structure-activity relationship) may correlate the structure and biological activity thus promoting enhanced study at the molecular level. Cheminformatics research such as ADMET (pharmacokinetics and toxicity filters) followed by QSAR analysis will report the potent leads and hasten the process. Consequently, the adoption of compounds from the same species may reduce the variation in electrottopological parameters in model development. Hemidesmus indicus called Indian sarsaparilla is accounted as an ayurvedic medicinal plant since ancient times. The terpene fraction of the plant is reported with antimicrobial and anti-inflammatory activities but the bioactive compound responsible is not precisely documented [5, 6]. Our study involves the isolation of bioactive fraction responsible for antimicrobial activity and to study the in silico electrottopological parameters and to optimize them as the better lead in antimicrobial drug discovery.

2. MATERIALS AND METHODS

Bioassay-guided fractionation was used to isolate the terpene fractions of Hemidesmus indicus roots. The root powder was extracted by soxhalation. The crude extract and fractions obtained by column chromatography were subjected to assay.
2.1. Preparation of Different Fractions

50 grams of the root powder was extracted with hydroalcoholic mixture of methanol (80%) 60 degree celsius for 5-6 hours. After completion, the extract was concentrated under reduced pressure using a rotary vacuum evaporator. The crude extract was further fractionated with n-hexane, chloroform, ethyl acetate, methanol, and water. Different fractions were subjected to phytochemical analysis followed by antimicrobial assays. The chloroform fraction was further fractionated using chloroform and benzene and the fractions were subjected to phytochemical analysis and thin layer chromatography studies [7].

2.2. Evaluation of Antibacterial Activity

Microbial strains such as *Staphylococcus aureus*, *Escherichia coli*, and *Aspergillus niger* were maintained at Centre for Biotechnology, Anna University, Chennai. Agar culture was standardised to $10^6$ cfu mL$^{-1}$. Agar diffusion assay was carried out to assess the antimicrobial activity. The terpene fractions were added and the plates were incubated at 37°C for 24 h (bacterial) and 28°C for 72 hrs during which activity was evidenced by the presence of a zone of inhibition surrounding the well.

2.3. Gas Chromatography-Mass Spectrometry

The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold - Perkin Elmer.

Turbomass 5.1 spectrometer with an Elite -1 (100% Dimethyl poly siloxane), 30 m × 0.25 m ID × 1 μm of the capillary column. Injection port temperature was ensured at 250°C and Helium flow rate at 1.5 ml/min. The samples were injected in split mode as 10:1. The mass spectral scan range was set at 40-700 (m/z). The MS start time was 3 min, and the end time was 35 min with solvent cut time as 3 min. Using computer searches on a NIST data library, The spectrum obtained in the data library were compared and identified.

2.4. Ligand and Proteins Preparation

The SYBYL 1.3 X was used as the workstation and thus for preparing ligands and proteins. The crystal structure of different receptors in bacteria such as D hydrofolate reductase-Bacillus (4NIR) and Threonyl-tRNA synthetase- *Staphylococcus aureus* (1NYR) Myristoyl transferase (4UWJ) was retrieved from PDB. Different ligands of *Hemidesmus indicus* such as Oleanen 3yl acetate (Chemspider ID 306426), Phthalic acid (Pubchem ID 1017), and Eicosane (Pubchem ID 8222) were retrieved and their geometries were stabilized. The interaction studies of the ligands with the different receptors were studied by docking protocol in SYBYL. Virtual screening based on best-fit ligand was done using ZINC and PUBCHEM databases. Drug-like and ADMET filters were applied to the reported ligands and used as an input for QSAR modelling.

The standard COMFA procedure as implemented in SYBYL 1.3 X was adopted to derive a correlation between the biological activity of a set of molecules and their 3d shape, electrostatic, and hydrogen bonding characteristics. Molecules with activities spanning about three log units of ki or ic50 values are required. In this study, we have considered the pic50 values of the non-toxic ligands for CoMFA analysis. It involves the sequential screening followed by optimisation using molecular mechanics. Molecules were included in the test and the training set. Compounds were selected randomly and models thus developed by minimum energy conformer based alignment. The steric and electrostatic fields were used as the deterministic feature in this study.

The binding of phthalic acid with target proteins such as Myristoyl transferase, Dihydroprotease synthase, and Threonyl tRNA synthase was to be analyzed by simulation for 10ns. Simulated annealing is a type of molecular dynamics experiments to obtain several different Version low energy conformation of a single molecule or to obtain several different low energy configurations of a system of molecules. at 100 K for 10 cycles [8].

3. RESULTS AND DISCUSSION

3.1. Preparation of Crude Extract

Crude Rev extract was prepared by soxhalation and the percentage yield was calculated. Phytochemical analysis revealed the presence of alkaloids, terpenoids, steroids, saponins, and glycosides. Crude extract was assessed for its antimicrobial activity.

3.2. Evaluation of Antibacterial Activity

The chloroform fraction was found to exert good antimicrobial activity in both bacteria and fungi. The fraction was again fractionated using chloroform and in column chromatography and the fractions were assessed for antimicrobial activity and fraction 4 was found to have good antibacterial and antifungal activities. Thus the fraction was further subjected to phytochemical analysis and thin layer chromatography, confirmed for the presence of terpenoids.

3.3. Gas Chromatography and Mass Spectrometry

The GC-MS chromatogram of the bioactive fraction of *Hemidesmus indicus* is shown. It was observed that the different peaks were obtained at different retention times. The highest peak at a retention time of 28 shows the presence of a triterpenoid- 12-oleanen-3-yl-acetate, the next major peak at a retention time of 15 shows the presence of phthalic acid and finally, the cluster of peaks from retention times ranging from 21 to 26 show the presence of coumarins (terpenoids) 12- oleanen 3yl acetate, Eicosane, Phthalic acid, and substituted alkanes as shown in Fig. (1).

3.4. Molecular Docking and QSAR Modelling

Water molecules and other ligands attached to the protein molecule were removed and the force field was applied in receptor cavities, wherein it shows the active sites of the receptor. Stabilization of the structure was performed to improve its biological activity and binding affinity. The
active sites were changed manually for every ligand and individually docked with the two receptors with the generation of dock score. After docking, suitable conformations: (i) the orientation of the docked conformation is in accordance with that of the ligand in crystal complex; and (ii) the conformation owns the highest C_score value. In the Surflex-Dock, the structures of ligands are flexible and the structure of the receptor is rigid.

The comparison between different (test and training) indicated that COMFA shows good predictive activities, thus acting as a predictive guide for designing new molecules. The visual inspection of the COMFA model will reveal the changes in substitution decreasing the activity. The yellow and green contours represent the steric activity of the compound. The yellow and green colours (Fig. 2) represent the steric activity of the compound. The yellow contours represent regions where steric bulk is not preferred in the compound i.e. small groups can be attached to these regions. The green contours represent regions where steric bulk is preferred, i.e. attachment of bulky groups will be favourable. The green contour at Carbon-20 and Sulphur-2 indicates that

![Graph showing GC MS analysis](image)

**Fig. (1).** GC MS analysis of the bioactive fraction of *Hemidesmus indicus.* (A higher resolution / colour version of this figure is available in the electronic copy of the article).

![Graph showing QSAR model](image)

**Fig. (2).** QSAR model developed from ligands of *Hemidesmus indicus.* (A higher resolution / colour version of this figure is available in the electronic copy of the article).
the substitution by bulkier groups will increase the activity of the molecule. The bright yellow contour at Carbon 16 and Carbon 26 indicates that the substitution by bulkier groups will decrease the activity of the molecule. This contour map gives us some general insight into the nature of the receptor-ligand binding region. The model was produced after 10ns of Molecular Dynamic simulation.

The residues found to interact with Phthalic acid were: TYR159, TYR333, TYR263, TYR39, LYS50, GLU33, ARG365, and LYS471. After the simulation, no newly interacted residues were found as shown in Fig. (3).

4. DISCUSSION

Drug discovery involves many reckoning steps towards the release of successful leads in the pharmaceutical industry [9]. The complexity existing in natural product chemistry and the synergistic and antagonistic nature of the phytoconstituents in the mixture impose and necessitate the involvement of different expertise to achieve success in their process. There is a broad and long gap in the integration of data, thereby integrating cheminformatics with plant science may repurpose the existing leads with better pharmacokinetic and pharmacological properties. Though in silico science reports several hits, lead optimization is hampered circumstantially in recent decades. Thus activity guided isolation was progressed with in silico QSAR model development using pharmacophore-based mapping may reveal putative unexplored ligands with better pharmacological properties.

CONCLUSION AND HIGHLIGHTS

The bioactive fraction of Hemidesmus indicus was separated from the extract of Hemidesmus indicus by sequential fractionation. The individual fractions were assessed for their antimicrobial potential.
The bioactive fraction was analysed by chromatography followed by GC-MS to identify the constituents using the data library.

The individual phytoconstituents were studied using force field analysis in 3D QSAR model development followed by molecular simulation studies.

Thus among the constituents, phthalic acid analogues were found to have a better binding with all the receptors. The same can be optimized using in vitro and in vivo studies.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES