A computational approach on the anti-biofilm effect of *Ocimum sanctum* bio-compounds against ptk of *Acinetobacter baumannii*

Abstract

Introduction: *Acinetobacter baumannii* is a gram negative coccobacilli often considered as a nosocomial pathogen and as an opportunistic pathogen in immunocompromised patients. It is considered to be multi-drug resistant and a potent bacteria forming vital biofilms. Ptk which is protein tyrosine kinase is a protein coding gene involved with the synthesis of capsular polysaccharide. *Ocimum sanctum* is a perennial plant belonging to the Lamiaceae family. Tulsi and holy basil are the common names of this plant. In-silico docking approach method is much more convenient and cost effective to assess the bioactive properties of the natural drugs against any target ligands.

Aim: The aim of the study was to assess the inhibitory effect of *Ocimum sanctum* bio-compounds against ptk of *Acinetobacter baumannii* using a computational approach.

Materials and Methods: Retrieval of the structure of ptk was followed by Ligand preparation and optimisation. Further drug likeliness was assessed using Molinspiration parameters, docking simulations and visualisation for the binding energy and hydrogen bonds.

Results: Among the bio compounds of *O.sanctum*, benzofuran is selected as an active inhibitory compound with -11.12 as its binding energy showing a high affinity.

Conclusion: The findings of the present study documents benzofuran as the promising candidate to design novel drugs from *O.sanctum* and to target the ptk of *A.baumannii*. However further experimental validation must be done to observe its efficacy and safety in the treatment of nosocomial infections caused by *A.baumannii*.

Keywords: Innovative in-silico; *O.sanctum*; novel ptk; *A.baumannii*; Benzofuran, environmental strains

Running title: Drug - ligand interactions of O.sanctum biocompounds with ptk of *A.baumannii*

Introduction

Acinetobacter baumannii is gram negative coccobacilli and may be considered as a hospital derived nosocomial pathogen and sometimes can be considered as an opportunistic pathogen in immunocompromised patients. *A.baumannii* is considered to be among the top six drug resistant microbes (1). The antimicrobial effect of different molecules can be used for targeting various nosocomial diseases (2). These molecules also find insight in implications to enhance immunity (3). Many modifications were recently found involved in the regulation of such as inflammatory and antitumor immune responses, antiviral immunity (4). Variations in the human genetic system are proven to affect disease progression and prognosis of the diseases (5). Exosomal microRNAs were found to be a promising tool in diagnosis of various systemic conditions (6). Removal of pathogens from the site of infection remains a confusing task which requests the use of antibiotics (7). A.baumannii is considered to be multi-drug resistant and a dangerous bacteria forming biofilm. *A.baumannii* models a unique property to maintain and exhibit a multidrug-resistant phenotype, further leading in complicating treatment (8). The natural habitat of the microbe is still not known. Prolonged hospital stay, weak immune system, chronic lung diseases, illness that requires use of hospital catheters and ventilators, forms some of the base etiological factors for the disease caused by *A.baumannii*. Its ability to survive on the artificial surfaces and resistance to desiccation and hospital environment is suspected to be favourable for the growth of *A.baumannii* due to constant use of antibiotics. Open wounds, catheters and breathing tubes pave the way for the entry of the microbe. Symptoms of this infection include pneumonia, meningitis, necrotising fasciitis and UTI infections. Previous studies stress the fact that molecular mechanisms guide the antimicrobial potential of drugs against complex pathogens (9). There are a wide range of virulence factors exhibited by *A.baumannii* such as phospholipases, outer membrane proteins, lipopolysaccharides, hemolytic factors, elastases and many more amongst which ptk gene is taken into an account. Ptk gene is taken as a gene of interest as it is a potent virulence factor of *A.baumannii*. The ptk gene which is protein tyrosine kinase is a protein coding gene involved with the synthesis of capsular polysaccharide. It is concentrated in local adhesions between cells growing in an extracellular matrix. Biofilm formation is one the important features of *A.baumannii* due to the existing niche and the chemical nature of antimicrobial agents. *A.baumannii* shows a variety of molecular mechanism actions which includes such as mutations, membrane permeability variations (10). The microbe has stealthily entered the oral cavity and acts as a potential pathogen by expressing various virulence factors (11).

As Siddha and Ayurveda are a vital part of Indian medicine, an arena of natural plants and herbs are used as antimicrobial agents against many microbes. These natural plants and herbs can be converted into pharmaceuticals and commercialized as they are easily available in abundant quantities. In a similar manner, here *Ocimum sanctum* commonly known as tulsi, the queen of herbs is considered to be the herb of interest. *Ocimum sanctum* is a perennial plant belonging to the Lamiaceae family. Tulsi and holy basil are the common names of this plant. It is considered for its aroma, traditional medicinal properties. It is a many branched subshrub with green leaves and strongly scented. This plant is widely used in day to day practice because of its easy availability. It is useful in the treatment of many diseases such as bronchitis, malaria, skin diseases and many more. Lately, it is also suggested for possessing antifertility, anticancer, antifungal, antimicrobial actions. The chemical constituents of tulsi consist of oleanolic acid, ursolic acid, eugenol, linalool, caryophyllene. The benzene extracts of various parts of the plant is useful in curing various ailments and eugenol which is one of the main chemical components acts on the immune system (12). Tulsi essential oil acts as a valuable topical antimicrobial agent for management of many skin diseases (13). Experimental validation can be time consuming, expensive and requires a lot of sources. This study is thus achieved with a computational approach for identifying each compound-ligand interaction and it is made easier. In-silico docking approach method is much more convenient and cost effective. The purpose of this practice is to give a tinge of how docking works to identify small flexible molecules to enormous protein structures. This method is extremely useful for finding potential binding sites and to discover novel molecules that possess the capacity to bind to a known site. Virtual screening and docking are employed in order to discover new medicines. The knowledge and expertise gained from the previous literature have been incorporated in the study design of this investigation (14–18) (19) (20) (21). The aim of this study was to identify the inhibitory effect of *Ocimum sanctum* bio-compounds against ptk of *Acinetobacter baumannii* using a computational approach (22) (23) .

Materials and Methods

Study setting

This is an observational in-silico study done in the Department of Microbiology, Saveetha Dental College and Hospital. Institutional approval for the research was obtained (SRB approval number: IHEC/SDC/UG-1907/21/158).

Retrieval of structure of ptk gene

The sequence of PTK from *Acinetobacter baumannii* was retrieved from NCBI database and the Biovia discovery studio visualiser was used to view the three dimensional structure of ptk gene (24). It was modeled using Swissmodel server using the template 3LA6 – A Chain.

AUTHOR HAS TO JUSTIFY THE TARGET PROTEIN STRUCTURE USED IN THIS STUDY; BECAUSE STRUCTURE NOT YET DEPOSITED IN PROTEIN DATA BANK (PDB).

Ligand preparation and optimisation

The structures of the bio-active derivatives of *Ocimum sanctum* were retrieved from the Pubchem database. The generated 3D structures were then optimised. 2D structure was drawn and optimized using ACD Chemsketch and saved in .mol format and converted to .pdb format using Open Label molecular converter tool.

Mol-inspiration assessment of the molecular properties of the selected compounds

The counts of hydrogen bond acceptors and donors in correlation to the membrane permeability and bio-availability of the compounds. The n-violation values of bioactive compounds are 0 satisfying Lipinski's Rule of 5. TPSA is a very useful descriptor used to characterize drug absorption and bioavailability, permeability through Caco-2 cells and transport across blood brain barriers. The characteristics of absorption, distribution, metabolism and elimination of the bio compounds of *Ocimum sanctum* were further analysed on the basis of ‘‘The Lipinski’s rule of five”.

Docking simulations

The Auto Dock tool was used for docking analysis to interpret the affinity between bio-compounds of *Ocimum sanctum* against ptk of *A. baumannii.*

Docking visualisation

Using Biovia Discovery Studio Visualizer, the hydrogen bond interaction between bio-compounds of *Ocimum sanctum* against ptk of *A. baumannii* were visualised. Further docking score assessments, binding affinities, molecular dynamics and energy simulations, the relative stabilities hydrogen interactions 2D diagram between ptk gene and bio compounds were evaluated.

Results

Structural retrieval of ptk from *A. baumannii*

The 3D structure of ptk gene was retrieved from Biovia discovery studio visualiser (Figure-1). The sequence of PTK from *Acinetobacter baumannii* was retrieved from NCBI database and its sequence Id was A0A171EWN0. The structure of PTK was not available in the PDB database. The modeled structure was found to be highly plausible as it had 44.53% sequence identity with that of the template. Moreover, the Ramachandran plot also showed 89.5% of residues in most favored regions and with no residues in disallowed regions (Figure-2).

Structural retrieval of the ligands from *Ocimum sanctum* bio compounds

Chemsketch was used for retrieving the 2D, 3D structures, its SMILES format of the ligands from *Ocimum sanctum* as shown in (Table-1).

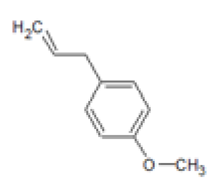
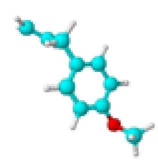
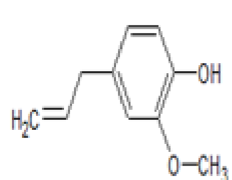
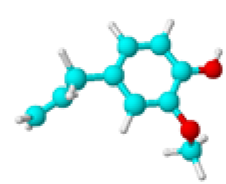
Drug properties by Molinspiration assessments:

The bioactivity score prediction, molecular weight, hydrogen bonds, and rotatable bonds of essential compounds of *Ocimum sanctum* against ptk gene of A. baumannii towards drug likeness was assessed and tabulated (Table 2).

Docking analysis for the drug-ligand interactions against ptk of *A. baumannii*

The bond interactions between the essential compounds from *Ocimum sanctum* and ptk gene of *A. baumannii* are shown in (Figure-3). The ptk gene interactions with compounds from *Ocimum sanctum are* shown in (Table 3). The number of hydrogen bonds, torsional energy and overall docking scores between the ligands and the drugs were evaluated (Table 4). The docking energies and interactions between the ptk gene and the *Ocimum sanctum* biocompounds were evaluated based on the Hydrogen bonds interactions, van der Waals interactions, π-r interactions/ amide-π stacked interactions/ π-cation interactions, alkyl/π-alkyl interactions and π-sulfur interaction.

Table-1- Table showing the the 2D, 3D structures of the ligands from *Ocimum sanctum*

Compound name	**2D**	**3D**
Estragole		
Eugenol		

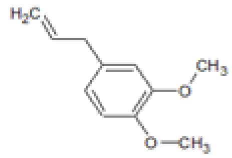
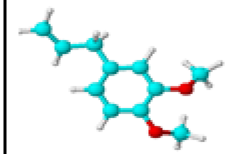
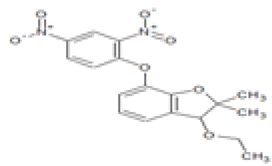
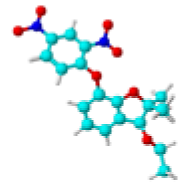
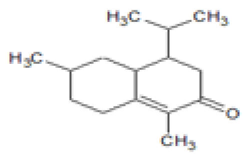
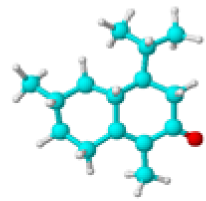
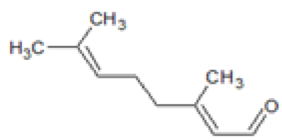
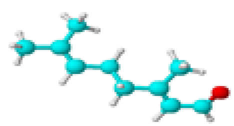
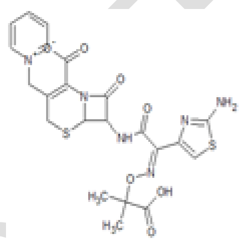
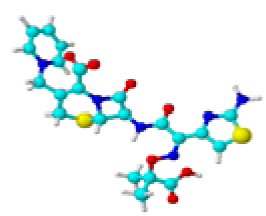
Methyleugenol		
Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl		
Hexahydro-1,6-dimethyl-4-(1-methylethyl)-		
Citral		
Ceftazidime		

Table-2- Table showing the molinspiration results of essential compounds of *Ocimum sanctum* against *ptk* of *A.baumannii*

Compounds	M.wt	Hydrogen Bond Donor	Hydrogen Bond Acceptor	miLogP	Rotatable bonds	nViolations	TPSA (Ǻ)	Volume	N atoms
Estragole	148.21	0	1	2.82	3	0	9.23	154.12	11
Eugenol	164.20	1	2	2.10	3	0	29.46	162.14	12

Methyl eugenol	18.47	0	2	2.41	0	0	18.47	179.67	13
Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl	374.35	0	9	4.49	6	0	119.35	318.05	27
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	220.36	0	1	4.66	1	0	17.07	238.11	16
Citral	152.24	0	1	3.65	4	0	17.07	169.74	11
Ceftazidime	546.59	4	13	-5.68	9	2	191.23	439.78	37

Table-3- Table showing the *Ptk* interactions with compounds from *Ocimum sanctum*

PTK docking with compounds	**Hydrogen bonds interactions**	**van der Waals interactions**	**π-σ interactions/ π-π T-shaped interactions/ amide-π stacked interactions**	**alkyl/π-alkyl interactions**	**Other interactions**
Estragole	ARG711	ILE516 GLN710 TYR721 ASN720 THR485 VAL486	-	ALA722 PRO487 (2) ILE709 (2) ARG711	-
Eugenol	SER552 (3) LYS551 GLU548	PRO547 VAL549 GLY550 PHE553 TYR580	-	LYS503	-
Methyleugenol	GLN710	ASN707	ILE709	ILE709	-

	ALA680	ARG678 ASP708 VAL468 ASN469 ASN720 THR485 SER471 ASP470		LEU706	
Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl	SER552 (2) LYS551(2) GLU548	ASP708 PHE553 SER504 SER489 GLN492 VAL549 PRO547	GLY550	-	**π** -cation LYS503
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	SER471	VAL468 ASN469 ASP470 ASP708 GLN710 ARG711 SER712 TYR719 ASN720	-	LEU706 ILE709(2) ALA680(2)	-
Citral	LYS551 SER552 (2)	GLU548 TYR580 GLN492 SER504 GLY550	PHE553	LYS503	-
Ceftazidime	ALA680 ARG480 LYS681 LYS681 ARG678 GLN710 GLN710 GLN710	ASP470 ASN469 VAL468		ALA680 LEU706 ILE709	

Table-4- Table showing the overall docking scores between the ligands and the drug.

PTK docking with compounds	Number of hydrogen bonds	Binding energy	Ligand efficiency	Intermolecular energy	vdW + Hbond + desolv Energy	Electrostatic energy	Torsional energy	Total internal Unbound
Estragole	1	-5.41	-0.5	-6.38	-6.27	-0.12	0.89	-0.22
Eugenol	5	-5.83	-0.49	-7.02	-6.68	-0.34	1.19	-0.34
Methyleugenol	2	-5.48	-0.42	-6.65	-6.25	-0.39	1.19	-0.44
Benzofuran, 7-(2,4-dinitrophenoxy)-3-ethoxy2,3-dihydro-2,2-dimethyl	5	-11.12	-0.41	-12.91	-10.23	-2.68	1.79	-0.87
Hexahydro-1,6-dimethyl-4- (1-methylethyl)-	1	-7.34	-0.46	-7.63	-7.7	0.06	0.3	-0.28
Citral	3	-5.18	-0.47	-6.38	-6.27	-0.11	1.19	-0.25
Ceftazidime	8	-7.63	-0.21	-10.91	-7.44	-3.47	3.28	-2.39

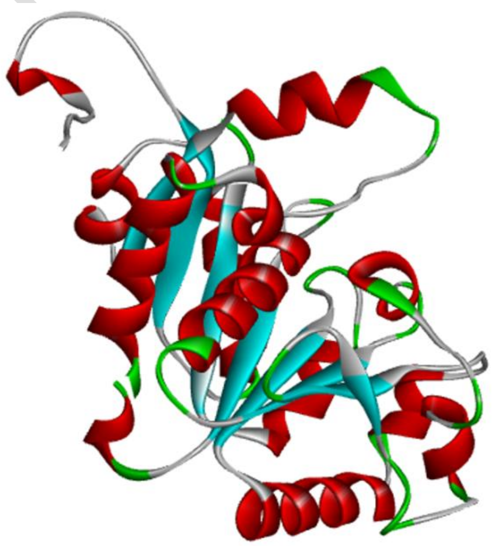


Figure 1: 3D Structure of *PTK* visualization using Biovia Discovery studio visualizer

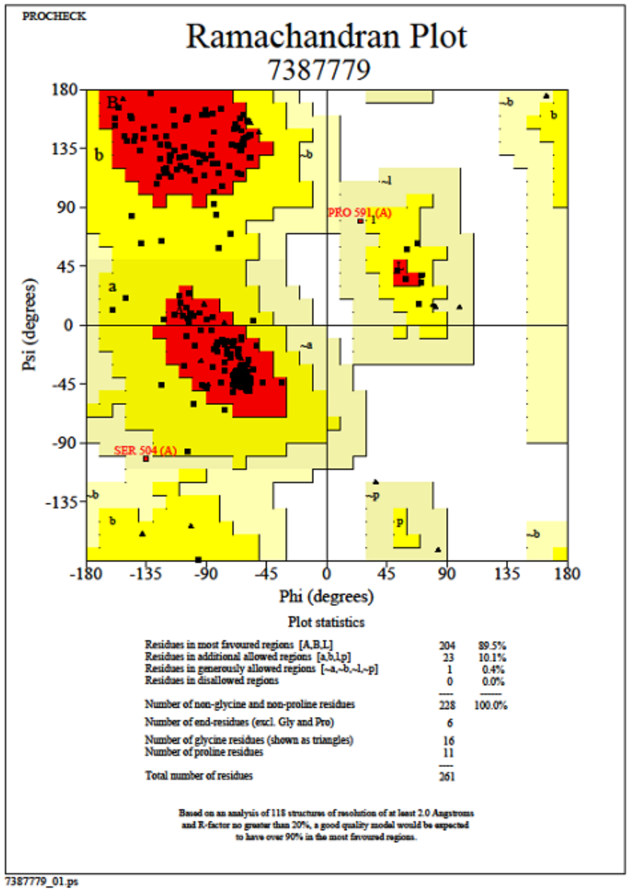


Figure 2: Ramachandran plot showed 89.5% of residues in most favored regions and with no residues in disallowed regions.

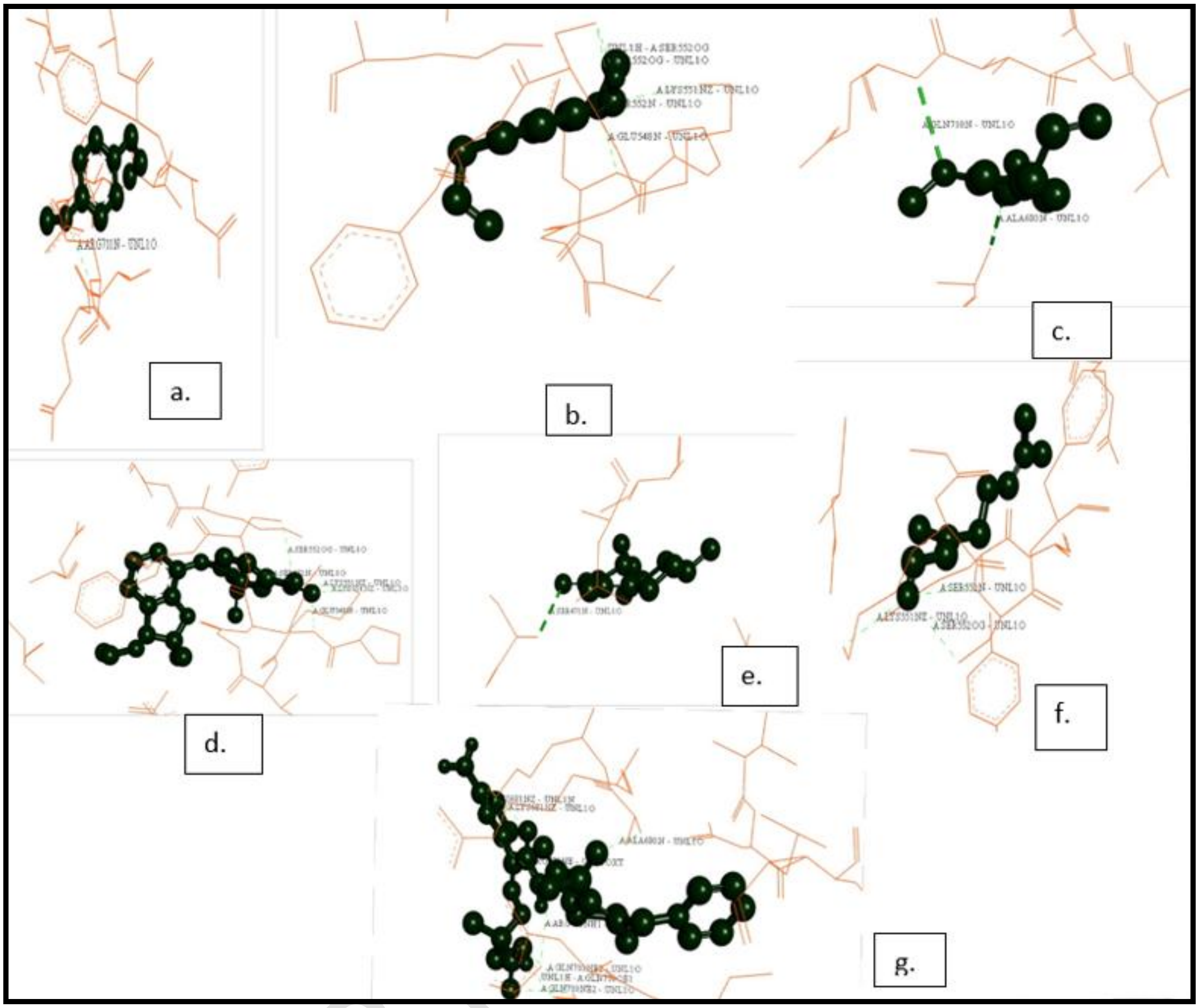


Figure-3- 3D diagrams showing the bond interactions between the essential compounds from *Ocimum sanctum* and *ptk* of *A. baumannii* a.Estragole b.Eugenol c.Methyleugenol d.Benzofuran, 7-(2,4- dinitrophenoxy)-3-ethoxy2,3- dihydro-2,2-dimethyl e.Hexahydro-1,6-dimethyl-4- (1-methylethyl)- f.Citral g.Ceftazidime

Discussion

A.baumannii has the ability to form biofilm by four steps which is attachment to the surface followed by formation of micro colony, maturation and colonisation. PTK is a potent virulence factor of *A.baumannii*. Previous research studies lack specific documentations on PTK. Computational based approaches seem to be of higher value in assessing the drug ligand interactions based docking studies. In a previous study, csgA protein of *A.baumannii* and the anti-biofilm activity of *A.indica* was assessed (25). However no earlier studies had documented the inhibitory effect of *Ocimum sanctum* biocompounds on ptk of *A.baumannii*.

Selection of essential compounds from *Ocimum sanctum* was based on references from earlier literatures (12). Eugenol which is an active and main constituent was found to occupy the major percentage of therapeutic use of Tulsi (26). The phenolic compounds of the herb were well-established with the help of spectroscopic methods leading to the identification of new compounds which had antioxidant and cyclooxygenase inhibitory activity (27). The pharmacological actions of *Ocimum sanctum* can be converted into standardized medicinal products which can be commercialised (28).

A.baumannii being a multidrug resistant and invasive pathogen, there are enormous possibilities for bio-compound interaction with the same that can be converted into drugs of pharmaceutical use (29). Carbapenemases which is one of the enzyme components of *A.baumannii* was discovered to increase and transform the species which paves way for identification of more potential medicines (30). Routine therapy enables the application of only fewer antibiotics as the species develops resistance towards many drugs. *A.baumannii* exhibited resistance by both phenotypic and genotypic characterisation methods (31).

In overall docking energies, ceftazidime has the highest number of hydrogen bonds and has highest avidity, however violating the Lipinsky's rule. Estragole has the lowest number of hydrogen bonds which shows that it has low avidity. The binding energy of ligands were analysed in the overall docking energies. The compound which possesses more negative value is said to have more affinity. In that case, amongst the bio-compounds of *O.sanctum*, benzofuran which is an active compound has -11.12 binding energy which shows that it has highest avidity. Citral has -5.18 binding energy which shows it has low avidity.

When the molecular weights of all the compounds were taken into account, ceftazidime had the highest molecular weight with 546.59 whereas methyl-eugenol had the lowest molecular weight with 18.47. Remaining compounds were found to have molecular weight ranging between 140 to

375. Resistance to ceftazidime is common among the clinical strains (32), thus suggesting the natural bio-compounds as the alternative source for treatment.

The TPSA value which is Topological Polar Surface Area acts as a major factor in deciding the importance of a bio-compound as it evaluates the oral bioavailability of drugs. The value should be <140Å. In the present study, it is notable that almost 6 out of 7 compounds have TPSA value less than 140 Å where ceftazidime has 191.23 Å. In the study by Sivaharini et al., caffeic acid had TPSA value < 140 Å and it was found to have best oral bioavailability (33). Computational based approaches thus hold good to predict the oral availability of the compounds to be designed as novel drugs (34, 35).

The limitations of the study was that it is done with a computational approach so as to obtain results in a lesser time and also found to be cost-effective. Further experimental validation can be performed with this study as reference to obtain more significant results for the drug ligand interactions.

Conclusion

The present study is undertaken to evaluate the inhibitory effect of the bio-compounds selected from *O.sanctum*. Computational approach on the same, documents the promising inhibitory effect of benzofuran that can efficiently target the ptk of *A.baumannii*. However further experimental validation must be done to observe its efficacy and safety in the treatment of nosocomial infections caused by *A.baumannii*.

NOTE:

The study highlights the efficacy of " Siddha and Ayurveda " which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

1. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related species . Vol. 94, Archives of Oral Biology. 2018. p. 93–8.

2. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020 Feb;43(2):153–4.

3. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol. 2020 Jun;17(6):668–9.

4. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol. 2020 May;17(5):550–1.

5. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? Front Immunol. 2020 May 27;11:1206.

6. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res. 2020 Jan;43(1):74–5.

7. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Nat Prod Res. 2021 Jun;35(11):1893–8.

8. Girija SA, Jayaseelan VP, Arumugam P. Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection. Acta Microbiol Immunol Hung. 2018 Dec 1;65(4):539–50.

9. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019 Dec;90(12):1441–8.

10. Padmaja S, Smiline Girija AS, Vijayashree Priyadharsini J. Frequency of Adhesive Virulence Factor fimH among the Clinical Isolates of Acinetobacter baumannii in India. Journal of Pharmaceutical Research International. 2020. p. 12–7.

11. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of Acinetobacter baumannii as an oro-dental pathogen and its drug resistance gene profile – An in silico approach. Vol. 4, Heliyon. 2018. p. e01051.

12. Prakash P, Gupta N. Therapeutic uses of Ocimum sanctum Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. Indian J Physiol Pharmacol. 2005 Apr;49(2):125–31.

13. Yamani HA, Pang EC, Mantri N, Deighton MA. Antimicrobial Activity of Tulsi (Ocimum tenuiflorum) Essential Oil and Their Major Constituents against Three Species of Bacteria. Front Microbiol. 2016 May 17;7:681.

14. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. Int J Paediatr Dent. 2021 May;31(3):436–41.

15. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? Int J Paediatr Dent. 2021 Mar;31(2):285–6.

16. Barma MD, Muthupandiyan I, Samuel SR, Amaechi BT. Inhibition of Streptococcus mutans, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. Arch Oral Biol. 2021 Jun;126:105132.

17. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci. 2020 Dec;15(4):562–3.

18. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent. 2020 Apr 1;18(1):379–86.

19. Jayaseelan VP, Paramasivam A. Emerging role of NET inhibitors in cardiovascular diseases. Hypertens Res. 2020 Dec;43(12):1459–61.

20. Iswarya Jaisankar A, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of A. baumannii and targeting with essential oil compounds from Azadirachta indica. Journal of King Saud University - Science. 2020 Dec 1;32(8):3380–7.

21. Girija AS. Fox3 (+) CD25 (+) CD4 (+) T-regulatory cells may transform the nCoV's final destiny to CNS! Journal of Medical Virology, 2020:9:5623-27.

22. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from Ganoderma lucidum: A computational study. pharmaceutical-sciences. 2020;82(2).\:1-7.

23. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with Murraya koengii bio-compounds: An in-silico approach. Acta Virol. 2020;64(1):93–9.

24. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from Porphyromonas gingivalis with the bioactive compounds from Rosmarinus officinalis. Asian Biomed . 2019 Oct 1;13(5):197–203.

25. Jaisankar AI, Smiline Girija AS, Gunasekaran S, Vijayashree Priyadharsini J. Molecular characterisation of csgA gene among ESBL strains of A. baumannii and targeting with essential oil compounds from Azadirachta indica . Vol. 32, Journal of King Saud University - Science. 2020. p. 3380–7.

26. Cohen MM. Tulsi - Ocimum sanctum: A herb for all reasons. J Ayurveda Integr Med. 2014 Oct;5(4):251–9.

27. Wangcharoen W, Morasuk W. Antioxidant capacity and phenolic content of holy basil. Songklanakarin J Sci Technol. 2007;

28. Singh D, Chaudhuri PK. A review on phytochemical and pharmacological properties of Holy basil (Ocimum sanctum L.) . Vol. 118, Industrial Crops and Products. 2018. p. 367–82.

29. Moubareck CA, Halat DH. Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. Vol. 9, Antibiotics. 2020. p. 119.

30. Pérez-Varela M, Corral J, Aranda J, Barbé J. Functional Characterization of AbaQ, a Novel Efflux Pump Mediating Quinolone Resistance in Acinetobacter baumannii. Antimicrob Agents Chemother. 2018 Sep;62(9).

31. A.S.Smiline Girija, J.Vijayashree, Paramasivam A. Prevalence of Acb and non-Acb complex in elderly population with urinary tract infection (UTI) . Vol. 76, Acta Clinica Belgica. 2021. p. 106–12.

32. Smiline ASG, Vijayashree JP, Paramasivam A. Molecular characterization of plasmid-encoded blaTEM, blaSHV and blaCTX-M among extended spectrum β-lactamases [ESBLs] producing Acinetobacter baumannii. Vol. 75, British Journal of Biomedical Science. 2018. p. 200–2.

33. Sivakumar S, Smiline Girija AS, Vijayashree Priyadharsini J. Evaluation of the inhibitory effect of caffeic acid and gallic acid on tetR and tetM efflux pumps mediating tetracycline resistance in Streptococcus sp., using computational approach. Vol. 32, Journal of King Saud University - Science. 2020. p. 904–9.

34. J.Sunitha, Swathy Krishna, R.Ananthalakshmi, J Sathiya Jeeva, A.S.Smiline Girija. Antimicrobial Effect of Leaves of Phyllanthus niruri and Solanum nigrum on Caries Causing Bacteria: An In vitro Study. Journal of Clinical and Diagnostic Research 2017;11 (6), KC01-KC04.

35. T Ushanthika, AS Smiline Girija, A Paramasivam, JV Priyadharsini. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. Natural product research 2021;35 (11), 1893-1898