Effect of *Azadirachta indica* Bio-Compounds against KpsM Protein of *Acinetobacter baumannii*

V. Thiru Kumaran[1], A. S. Smiline Girija[2*], P. P. Sankar Ganesh[2] and J. Vijayashree Priyadharshini[2]

[1]Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, India.
[2]Department of Microbiology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-600077, Tamilnadu, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author VTK carried out the literature search, data collection, data analysis and manuscript writing. Author ASSG conceived the study, participated in its design and coordinated and provided guidance to draft the manuscript. All authors have equally contributed in developing the manuscript.

ABSTRACT

Background: *Acinetobacter baumannii* was considered as a low priority pathogen earlier, and is been now reported as a priority pathogen causing nosocomial infections. Selection of natural compounds to target the organism is the need of the hour.
Aim: This study is aimed to target the KpsM protein of *A. baumannii* with the bio-compounds from *Azadirachta indica* using in-silico docking analysis.
Materials and Methods: KpsM protein was retrieved and optimisation of protein was done. After that optimization and ligand preparation was carried out. It was continued by molinspiration assessment of the molecular properties of selected compounds. It was followed by docking simulation and docking visualisation.
Results: Out of the 7 compounds of *Azadirachta indica,* dihydro diisoeugenol is the best compound to act on the KpsM protein of *Acinetobacter baumannii* and a binding energy of -6.83Kcal/Mol.

_____

Corresponding author: E-mail: smilinegirija.sdc@saveetha.com;

Conclusion: The findings of the study reports isoeugenol with more binding energy than other compounds towards the selected protein KpsM of *Acinetobacter baumannii*. However it requires further experimental studies to understand the mechanism of its actions and safety.

1. INTRODUCTION

Acinetobacter baumannii can cause recalcitrant infections of the urinary tract, lungs or in wounds and yet in different components of the body [1]. They additionally colonize or board an affected person without inflicting infections or symptoms, particularly through droplets or open wounds [2,3]. *A. baumannii* is a growing and foremost problem surviving in dry environments and is dreadful for its fast-spreading nature. It is multi-drug resistant and, typically to the last group of drugs, the carbapenems. *A.baumannii* is a topic of concern for its multidrug-resistant property as it has emerged as a nosocomial pathogen, with the capability to rival MRSA. *A.baumannii* is categorised under the ESKAPE group of pathogens and belongs to the ACB complex with an extensive property of drug resistance [4].

Many vital virulent genes are attributed for the virulence and resistance property viz., csuE, pgaB, KpsM, epsA, ptk, bfmS and ompA. 98% of the isolates possess more than four biofilm genes. The hydrophobicity profile of KpsM shows that it is a quintessential membrane protein, and is capable of binding to ATP-binding domain. KpsM and KpsT encompass a number of organic processes, such as membrane transport, signal transduction etc. Due to the severity of the multi-drug resistance and biofilm formation, most of the antibiotics are unresponsive for the treatment of *A. baumannii* associated systemic infections [5–6].

In this view, the application of alternative medication would aid in the eradication of *A. baumannii*. Among many established herbal drugs, *Azadirachta indica* is moreover a herb that is commonly known as Neem. The medicinal utilities had been defined in particular for neem leaf. Neem leaf and its parts had been tested to exhibit immunomodulatory, anti-inflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties. The extract comes from the seeds of the tree and has many diverse conventional uses [7]. The most crucial lively parts are azadirachtin and the others include potent compounds like nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin. Neem leaves encompass protein, carbohydrates, minerals, calcium, phosphorus, nutrition C, carotene etc. Drugs from such natural sources can be potent candidates for drug design and the application of bioinformatic tools and servers can be used to evaluate their interactions with the target proteins. The present study was thus undertaken to assess the drug ligand interactions of 7 different compounds from neem with KpsM protein of *A.baumannii* using computational analysis [8, 9].

2. MATERIALS AND METHODS

2.1 Study Setting

This was an observational *in-silico* study done in the Department of Microbiology, Saveetha Dental College and Hospital. Institutional approval for the research was obtained and the SRB approval number is IHEC/SDC/UG-1943/21/150.

2.2 Retrieval of KpsM Protein and Optimisation

The crystal shape of KpsM protein turned into received from the RCSB protein records bank. The optimisation of crystal shape of KpsM is performed through the addition of hydrogen atoms. Kollman united atoms pressure subject turned into used to assign digital expenses to the protein atoms which turned into performed withinside the AutoDock device and the RASMOL device turned into used for the visualisation of 3 dimensional shape of KpsM protein.

2.3 Ligand Instruction and Optimisation

The systems of the bio-energetic derivatives of *A. indica* have been received from the chemsketch software. The generated 3-D structures have been then optimised. The decided ligands have passed through next conversions through an open label molecular converter program. Then they have been stored in protein data base (PDB) format.

2.4 Molinspiration Evaluation

The counts of hydrogen bond acceptors and donors on the subject of the membrane permeability and bioavailability of the logP for partition coefficient were assessed using the molinspiration evaluation program.

2.5 Docking Simulations

The Auto Dock tool was used for docking evaluation to interpret the affinity among bio-compounds of *A. indica* towards the KpsM protein of *A. baumannii*.

2.6 Docking Visualisation

Using Discovery studio visualiser, the hydrogen bond interaction among the bio-compounds of *A. indica* towards KpsM of *A. baumannii* have been visualised. Using similarly docking rating assessments, binding affinities, molecular dynamics and electricity simulations, the relative stabilities have been evaluated.

3. RESULTS

3.1 Structural Retrieval of the KpsM Protein from *A. baumannii*

The sequence of KPSM from *Acinetobacter baumannii* was retrieved from Uniprot database and its sequence id was SVK37967.1. The structure of KPSM was not available in PDB database. Hence it was modeled using Swissmodel server using the template 6OIH– B Chain. The modeled structure was evaluated using SAVES-PROCHECK server, it was found to be a good quality model as 95.8% of the residue falls in the most favored region and no residues fall in the disallowed region.

The version became fairly viable with 100% series identification with the template. Besides, the Ramachandran plot confirmed 95.8% of residues in maximum favoured areas and without any residues in disallowed areas. The 3d shape of csgA visualized the use of RASMOL with the crimson color denoting the alpha-helix, yellow arrow denoting the beta sheets and white color denoting the turns.

3.2 Molinspiration Evaluation Closer to Drug Likeness

Based at the calculation of the ion channel modulation, GPCR ligand, nuclear receptor ligand, kinase inhibitor, enzyme inhibition and protease inhibition, the bioactivity rating prediction of essential additives of *A. indica* in opposition to KpsM of *A. baumannii* closer to drug likeness changed into assessed and tabulated (Table 1).
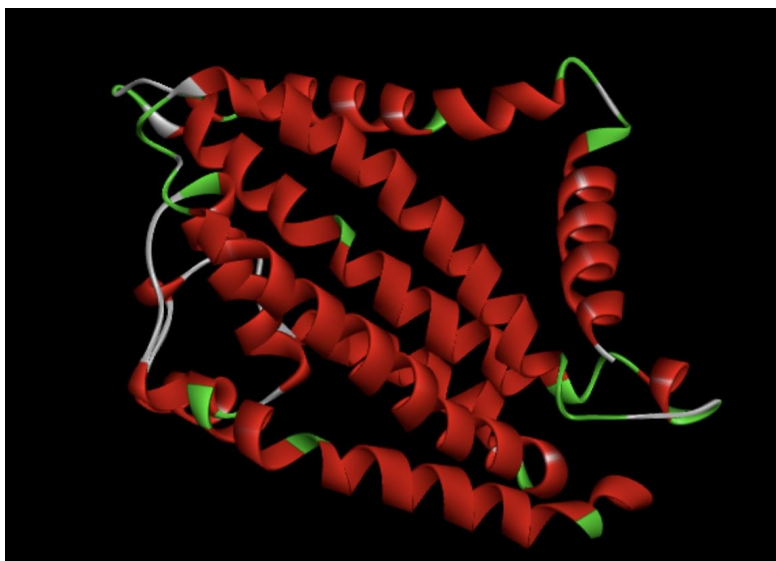

Fig. 1. Showing the 3D structure of KpsM of A.baumannii visualised by RASMOL

Table 1. Showing the molinspiration evaluations for drug-likeness property

Compounds	M.wt	Hydrogen Bond Donor	Hydrogen Bond Acceptor	miLogP	Rotatable bonds	nViolations	TPSA (Å)	Volume	N atoms
Imidazole-2-carboxylic acid, 4-methyl-	126.11	2	4	-0.17	1	0	65.98	104.44	9
Bis(2-propyl pentyl) phthalate	390.56	0	4	8.04	16	1	52.61	407.90	28
Dihydrodi Isoeugenol	326.39	1	4	4.10	4	0	47.93	306.90	24
4-Hydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	298.30	0	6	3.35	5	0	76.66	259.58	22
MethylEthyl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-5,6,7-tetrahydro indole-2-carboxylate	355.43	1	5	4.54	6	0	68.40	335.84	26
Ethyl 6,8-difluoro-4-hydroxyquinoline-3-carboxylate	253.20	1	4	0.10	3	0	59.17	203.20	18
Ceftazidime	546.59	4	13	–5.68	9	2	191.23	439.78	37

Table 2. Showing the drug - ligand interactions with the associated binding energies and hydrogen bonds

| KPSM docking with compounds	Number of hydrogen bonds	Binding energy	Ligand efficiency	Intermolecular energy	vdW + Hbond + desolv Energy	Electrostatic energy	Torsional energy	Total internal Unbound
Imidazole-2-carboxylic acid, 4-methyl-	6	-4.44	-0.49	-5.04	-2.7	-2.33	0.6	-0.56
Bis(2-propyl pentyl) phthalate	NIL	-4.8	-0.17	-9.57	-9.55	-0.22	4.77	-2.77
Dihydrodi Isoeugenol	NIL	-6.83	-0.28	-8.32	-8.35	-0.03	1.49	-1.18
4-Hydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	3	-6.78	-0.31	-8.27	-7.11	-1.17	1.49	-0.45
MethylEthyl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-5,6,7-tetrahydro indole-2-carboxylate	2	-6.68	-0.26	-8.47	-8.43	-0.04	1.79	-1.19
Ethyl 6,8-difluoro-4-hydroxyquinoline-3-carboxylate	3	-5.43	-0.3	-6.33	-6.27	-0.06	0.89	-0.08
Ceftazidime	6	-6.92	-0.19	-10.2	-7.53	-2.66	3.28	-3.09
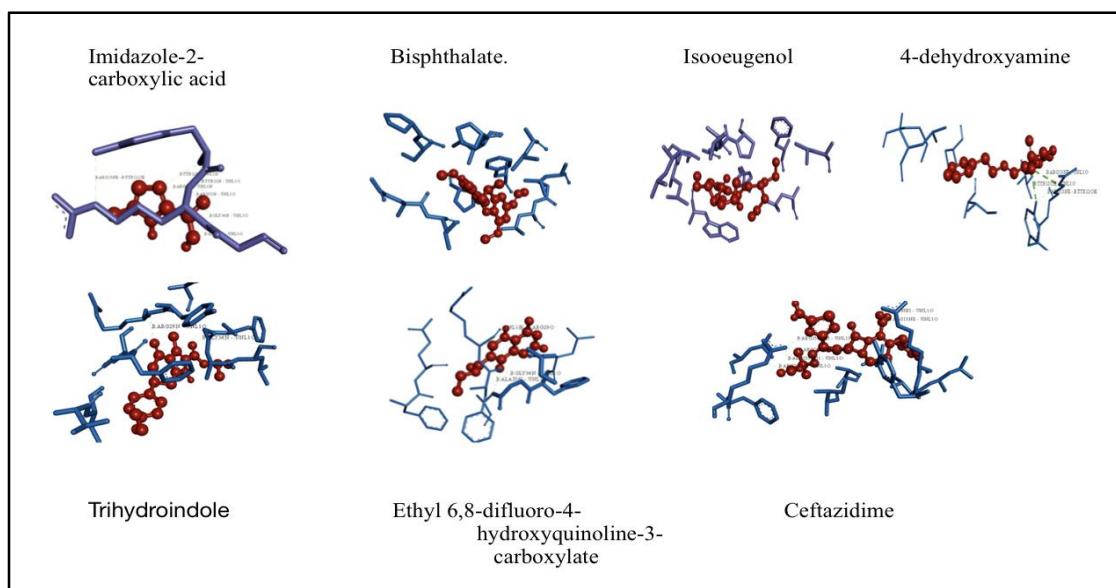
Fig. 2. Showing the docked molecules of the interactions of KpsMt and the compounds selected from *A. indica*

3.3 Docking Evaluation of the *A. indica* Derivatives towards KpsM of *A. baumannii*

LGA turned into used for choosing the first-class conformers. The bond inter- moves among the vital compounds from *A. indica* and KpsM of *A. baumannii* with inside the stick version via a means of discovery studio visualisations among the chosen compounds are shown (Fig. 1). The KpsM protein interactions with biolively compounds from *A. indica* are shown. The docking scores, quantity of hydrogen bonds formed, torsional strength among the ligands and the medication have been recorded (Table 2).

4. DISCUSSION

The amazing virulence component of *A. baumannii* is its cap potential to shape biofilms in a 4 fundamental step manner via., attachment of micro organism to the surface, formation of micro-colony, maturation of biofilms and compounds for the in-vitro analysis. The oil handled and the oil untreated businesses on assessment confirmed anti-biofilm impact significantly. Biofilm based assays have been documented earlier to report on the virulence genes involved in the pathogenesis of *A. baumannii*. Earlier research had documented the effect of neem compounds against ESBL producing strains of *A. baumannii* [10, 11]. In-

silico based computational approach is useful in the prediction of virulence genes and proteins, and can be best targeted by natural bio-active compounds [12] and also for the non-antibiotic drugs [13]. Bio-informatics tools and databases are easier to perform and can be best implemented for molecular studies [14] oral pathogens too [15], systemic diseases [16, 17] and also for pandemic associated studies [18]. Computational platform of tools also holds promising for the theragnostic detections [19] and for the selection of targets from fastidious organisms as well [20, 21].

Selection of the bioactive compounds from *A. indica* for the present investigation was done based on previous literature. The vital oil compounds from *A. indica* have been selected for in-silico assessments, because of the hydrophobic biomolecules and can be implemented as nano-formulations. Crude extracts have shown good activity however purification processes need to be done. With the resources of the computational bioinformatics equipment and databases, it is easier to evaluate the drug-ligand interactions. The pose, ligand – receptor properties and the molinspiration assessments hold good for the evaluation of the antimicrobial efficacy of vital compounds from neem.

Comparing the molecular weight of all of the compounds, imidazole-2-carboxylic acid

possesses the least molecular weight of 126. bis (2-propyl pentyl) phthalate ester possesses the better molecular weight of 390.36 that correlates with earlier reports . Other compounds confirmed a molecular weight ranging among >200 daltons. Assessments at the hydrogen bonds donor and acceptor property, bis (2- propyl pentyl) phthalate ester had the best wide variety of rotatable bonds of approximately sixteen collectively with the best miLogP cost of 0.17. The TPSA cost (Topological Polar Surface Area) of a compound is a vital evaluation, because it attributes to the oral bio-availabilty of medicine which ought to be <140>.

Evaluation of the general docking energies confirmed that imidazole-2-carboxylic acid were given the best range of hydrogen bonds even as bis(2-propyl pentyl) phthalate has were given the least. Trihydroindole indicates the least binding electricity of –9.39 while bis(2- propyl pentyl) phthalate indicates about –four.07. Dehydrodiisoeugenol possessed a best inhibition consistent while imidazole-2- carboxylic acid confirmed the least inhibition consistent. Ligand efficiency, electrostatic and torsional electricity had been observed to be more in bis (2-propyl pentyl) phthalate ester. We also can infer from the general interplay that imidazole-2-carboxylic acid confirmed 7 hydrogen bond interactions, four van der waals interactions, 1 p –r interplay and 1 p – alkyl interplay which states the stabilization of the binding structures. This changed into accompanied via way of means of 3-Quinolinecarboxylic acid with five hydrogen bonds and bis(2-propyl pentyl) phthalate ester displaying hydrogen bond interplay. Trihydroindole has were given the best Vanderwaal interplay accompanied via way of means of bis (2-propyl pentyl) phthalate. Dihydro diisoeugenol confirmed 2p–r interactions in association with p– alkyl interactions had been observed to be more in dihydroindole. On the opposite hand, handiest 3-Quinoline carboxylic acid confirmed p–sulfur interactions. Dihydro diisoeugenol is considered as the best compound with the binding energy of -6.83. The limitation of the study is that it was conducted as an in-silico based observation. Thus future prospects are set with evaluating the antimicrobial activity using in-vitro and in-vivo studies [12–23]. *A. baumannii* was selected as the study strains as it is one of the predominant nosocomial pathogen with multi-drug resistant property [24, 25]. Selection of bio-active compounds from natural sources and the drug ligand interactions in selecting the compounds of drug design is also promising [26, 27]. Apart from natural sources it can also be applied for the selection of compounds from marine sources and also for the selection of drug targets [28-32].

5. CONCLUSION

The findings of the study report dihydro diisoeugenol with more binding energy in comparison to the other compounds towards the selected protein KpsM of *Acinetobacter baumannii.* The inhibition effect has to be confirmed using the bio-assays further for the drug design and evaluation. So this compound can be considered as the best candidate to design as a novel drug and to treat *A. baumannii* infections. However further experimental validation must be done for its efficacy and safety through *in-vitro* and *in-vivo* studies.

DISCLAIMER

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.

FUNDING

The present study was supported by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences [SIMATS], Saveetha University and Sri Thirumalaipathy Transport.

NOTE

The study highlights the efficacy of "herbal" 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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mikulandra S. The effect of the methanol leaf extract of the plant *Azadirachta indica* (Neem) on the most common bacterial causes of ventilator associated pneumonia; 2017.
2. Shin B, Park W. Synergistic effect of oleanolic acid on aminoglycoside antibiotics against *Acinetobacter baumannii*. PLOS ONE. 2015;10:e0137751.
3. Hubble Bartholomew. Augmenting the Activity of *Macrolide adjuvants* against *Acinetobacter baumannii*. ACS medical Chem Letters. 2020;11(9):1723-31.
4. Berk E. In vitro synergistic activity of colistin and daptomycin combination against carbapenem-resistant *Acinetobacter baumannii*. International Journal of Ayurvedic and Herbal Medicine. 2020;562-68.
5. Cabral M. Protective efficacy of a D-glutamate auxotrophic live vaccine against *Acinetobacter baumannii* acute lethal infection. International journal of herbal medicines. 2020;6:1-3.
6. Palacios-Can FJ. QSPR model for the prediction of new antibacterial molecules against *Acinetobacter baumannii*. SAGE journal of medicines. 2020;13:431
7. Shahid F, Ashraf ST, Ali A. Reverse vaccinology approach to potential vaccine candidates against *Acinetobacter baumannii*. Methods in Molecular Biology. 2019;329–36.
8. Prasadacharyulu DCS, Prasadacharyulu CS, Associate Professor of Biochemistry P. V. K. N. Govt. College, Pradesh CA, India. Therapeutic Potentials of Bioactive Compounds of Azadiracta Indica (Neem) to Treat Ailments of Humanbeing. International Journal of Ayurvedic and Herbal Medicine; 2019.
9. Kamolvit W. Molecular epidemiology and insights into the genomes of Acinetobacter calcoaceticus - Acinetobacter baumannii complex. 10.14264/uql.2015.970 Corpus ID: 86185662.
10. Kırmusaoğlu S. Bacterial pathogenesis and antibacterial control. BoD – Books on Demand; 2018;154.
11. Mendez-Vilas A. Science and technology against microbial pathogens: Research, development and evaluation. World Scientific. 2011;448.
12. 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;93–8.
13. Vijayashree Priyadharsini J. *In silico* validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. J Periodontol. 2019;90(12):1441–8.
14. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): A promising new molecular target in hypertension and cardiovascular diseases. Hypertens Res. 2020;43(2):153–4.
15. 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. Heliyon. 2018;4(12):e01051.
16. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. Cell Mol Immunol. 2020;17(6):668–9.
17. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. Cell Mol Immunol. 2020 May;17(5):550–1.
18. 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;11:1206.
19. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. Hypertens Res. 2020;43(1):74–5.
20. 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;35(11):1893–8.
21. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from *Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis*. Asian Biomed. 2019;13(5):197–203.
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).

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. Sivakumar S, Girija ASS, Priyadharsini JV. 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. Journal of King Saud University-Science. 32(1):904-909.

25. Smiline Girija AS, Vijayashree Priyadhasini J. Plasmid encoded tet-A and tet-B mediated tetracycline, doxycycline and minocycline resistance among *Acinetobacter baumannii* isolated from urine samples. Roum Arch of Microbiol and Immunol. 76(3-4):134-140.

26. Anchana SR, Girija SAS, Gunasekaran S, Priyadharsini VJ. Detection of csgA gene in carbapenem-resistant *Acinetobacter baumannii* strains and targeting with Ocimum sanctum biocompounds. Iranian Journal of Basic Medical Sciences. 24(5):690.

27. Vaishnavi VP, Preetha Chaly S, Smiline Girija AS, Raghuraman R, Pandisuba K. Antimicrobial activity of Gotukola leaves and neem leaves: A comparative in-vitro study. Int J of Ayurveda and Holisitc Medicine. 3(3):11-15.

28. Smiline Girija AS, Duraipandiyan V, Pandi Suba K, Hariprasad G. Assessment of the anti- HBV like property of BEHP – a phthalate derivative and its in-silico characterization. BMC Infectious Diseases. 14(Suppl 3):8.

29. Smiline Girija AS, Pandi Suba K. BEHP – a phthalate derivative characterized form the south Indian squid and its anti-HCV like property – An in-vitro and in-silico analysis. International Journal of Pharma and Bio Sciences. 6(1B):401–410.

30. Smiline Girija AS, Gnanendra S, Paramasivam A, Priyadharsini JV. Delineating the potential targets of thymoquinone in ESKAPE pathogens using a computational approach. In Silico Pharmacology. 9(1):1-11.

31. 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;32(8): 3380–7.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74430