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Targeting Omp-A Protein of Acinetobacter Baumannii with the Bio-Active Compounds from Azadirachta Indica - an *in-silico* Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author RN did the literature search, data collection, analysis, manuscript writing. Author ASSG did the study design, data verification, manuscript drafting. Author PSG did the manuscript editing and revision. Author JVP did the validation of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Acinetobacter baumannii i*s a gram negative bacterium which is typically short, round, coccobacillus and was named after the bacteriologist Paul Baumann. It is an emerging dental pathogen since it acquires drug resistance and expression of several virulence genes. It is an opportunistic pathogen in humans, affecting people with compromised immune systems. *Acinetobacter baumannii* is an arising nosocomial microorganism causing serious complications because of the propensity of its multi-drug resistant property.
Aim: The aim of the present study was to target omp-A protein of *Acinetobacter baumannii* with the bio active compounds from *Azadirachta indica* an in-silico approach.
Materials and Methods: The crystal structure of ompA protein was obtained from the PDB protein data bank. The structures of the bio-active derivatives of *A. indica* were obtained from the chemsketch software. The generated 3D structures were then optimised. Auto Dock instrument

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was utilized for docking investigation to interpret the affinity between bio-compounds of A. indica against ompA protein of *A. baumannii*.

Results: The 3D crystal structure of OmpA-like domain from *A.baumannii* was retrieved from PDB database and its PDB ID was 3TD3 – A chain. 3D Structure of OmpA visualization using Biovia-Discovery studio visualizer. The 2D structure of compounds from Azadirachta indica was drawn using ACD chemsketch and saved in MDL-mol format and converted to PDB format using open babel converter. The final docked structures for the drug ligand interactions were assessed for their binding energies and hydrogen bonds.

Conclusion: The present study had achieved the anti-biofilm inhibitory effect of imidazole-2-carboxylic acid from *A. indica* exhibiting a great interaction between activity with ompA utilizing computational investigation.

Keywords: Acinetobacter baumannii; Azadirachta indica; novel ompA protein; docking.

1. INTRODUCTION

Acinetobacter baumannii is a gram negative bacterium which is typically short, round, coccobacillus and can cause opportunistic infections in humans, affecting people with compromised immune systems [1]. *Acinetobacter baumannii* is an arising nosocomial microorganism causing serious complications because of the inclination of its multi-drug safe property. Motility in *A. baumannii* may be because of the discharge of exopolysaccharide, making a film of high-molecular-weight sugar binds behind the bacterium initiate the infections in host tissues. *A. baumannii* is a part of the ACB complex comprising the most virulent members of the genus [2]. Carbapenems are infused as the last medication of choice for treating severe nosocomial infections caused by multidrug-resistant *Acinetobacter baumannii* strains [3]. It is presently an overall issue that metallo-β-lactamases (MBLs) as carbapenem-hydrolyzing chemicals as the significant medication for infections associated with biofilms.

Outer membrane proteins or OMP's are a class of unique integral membrane proteins anchored in the cell membrane, whose β-barrel structures were framed by 8 to 26 strands [4]. There are huge, expanded circles between the strands on the extracellular side and short circles on the periplasmic side. These attributes give OMPs high security in layer and ability to battle against brutal conditions. Albeit, diverse OMP's have various groupings and capacities, they share comparative design and natural properties. OMP's of microorganisms comprise multiple strands, and critically the capacity and stand shear number rely upon their successions [5]. Several resistance mechanisms contribute to the multidrug resistance (MDR) aggregate in *A. baumannii* such as diminished external layer protein (OMP) porousness, overexpression of efflux siphons, and procurement of hereditary components conveying opposition determinants, for example, plasmids, integrons, transposons, and obstruction islands. Gram-negative bacteria normally show assorted porins in their external layer that take an interest in the cell porosity, outer membrane protein A (OmpA) being the most abundant [6]. Detection and molecular diagnosis of these genes are performed routinely by PCR, specific multiplex PCR, and multi locus sequence typing. Adhesion can be a basic determinant of virulence for bacteria. The capacity to attach to cells permits microorganisms to co-operate with them differently, regardless of whether by type III discharge framework or basically by hanging on against the predominant development of liquids. Outer membrane protein A (OmpA) has been demonstrated to be engaged with the adherence of *A. baumannii* to epithelial cells [7]. This permits the bacteria to attack the cells through the zipper mechanism. The protein additionally appeared to confine to the mitochondria of epithelial cells and cause putrefaction by animating the creation of responsive oxygen species [8].Our team has extensive knowledge and research experience that has translate into high quality publications[9–13]

Azadirachta indica, normally known as neem, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae. It is one of the two species in the genus *Azadirachta*, and is local to the Indian subcontinent. It is ordinarily grown in tropical and semi-tropical districts. Components of *Azadirachta indica* include isomeldenin, nimbin, nimbinene, 6-desacetyl nimbinin, nimbandiol, immobile, niacinol, quercetin, and beta-sitosterol [14]. It has antibacterial activity against both *Staphylococcus aureus* and MRSA with greatest zones of inhibition. Compared to the in-vitro bio-

assays, the in-silico docking approach was less time-consuming and more easier. The protocol was designed as per the previous literatures and based on the expertise of our studies done earlier [15–21]. The present investigation is thus designed to evaluate the bio-compounds from *Azadirachta Indica* to target ompA protein of *A. baumannii.*

2. MATERIALS AND METHODS

2.1 Study Setting

The present study 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 number is IHEC/SDC/UG-1992/21/153)

2.2 Retrieval of Ompa and Protein Optimization

The crystal structure of ompA protein was obtained from the PDB protein data bank (Fig.1). The optimisation of crystal structure of ompA is done by the addition of hydrogen atoms. Kollman united atoms force field was used to assign electronic charges to the protein atoms which was done in AutoDock tool – 1.5.6 and the RASMOL tool was used for the visualisation of three dimensional structure of ompA protein.

2.3 Ligand Preparation and Optimization

The structures of the bio-active derivatives of *A. indica* were obtained from the chemsketch software. The generated 3D structures were then optimised (Fig. 2). The selected ligands were subjected to subsequent conversions by an open label molecular converter program. They were then saved in PDB format. The selected ligands were further saved in a mol file.

2.4 Molinspiration Appraisal of the Molecular Properties of the Selected Compounds

The counts of hydrogen bond acceptors and donor according to the membrane penetrability and bioavailability of the compounds, logP for partition coefficient, molecular weight of compounds of the essential molecular descriptors were evaluated with the assistance of molinspiration appraisal program. The characters of absorption, distribution, metabolism and elimination of the selected bio compounds were additionally assessed based on "The Lipinski's standard of five".

2.5 Docking Simulations

Auto-Dock tool was utilized for docking investigation to interpret the affinity between bio-compounds of *A. indica* against ompA protein of *A. baumannii.*

2.6 Docking Visualisation

Utilizing the Discovery studio visualiser, the hydrogen bond connection between the bio-compounds of *A. indica* against ompA of *A. baumannii* were visualised. With additional docking score appraisals, binding affinities, molecular-atomic elements and energy stimulation, the relative stabilities were assessed.

3. RESULTS

3.1 OmpA Structure Retrieval

The 3D crystal structure of OmpA-like domain from *Acinetobacter baumannii* was retrieved from PDB database and its PDB id was 3TD3 – A chain. 3D Structure of OmpA visualization using Biovia Discovery studio visualizer (Fig. 1).

3.2 Bioactive Compounds from Azadirachta Indica

The 2D structure of compounds from *Azadirachta indica* was drawn using ACD chemsketch and saved in MDL-mol format and converted to PDB format using open babel converter (Table 1).

3.3 Drug likeliness Properties Calculation using MOLINSPIRATION

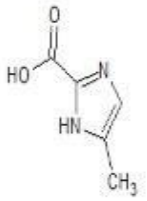
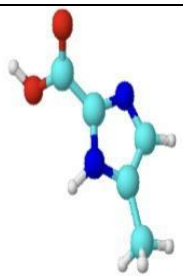
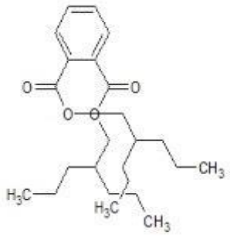
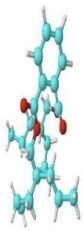
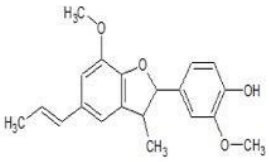
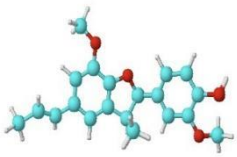
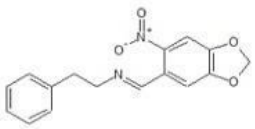
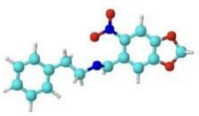
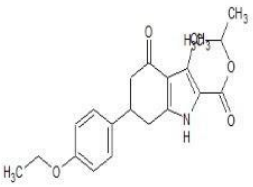
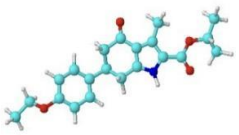
From the molinspiration results, except Bis (2-propyl pentyl) phthalate showing one violation, all the other compounds show violation values of the bioactive compounds as 0. Hence all molecules satisfy Lipinski's Rule of 5. Control drug Ceftazidime showed two violations (Table 2).

3.4 Docking Analysis of the *A. indica* Derivatives against Ompa of *A. baumannii*

The bond interactions between the specific compounds from *A. indica* and ompA of *A. baumannii* in the stick model by discovery studio visualisations between the selected compounds

are shown in Fig. 2. The ompA protein interactions with bio-active compounds from *A. indica* are shown in Table 3. The docking scores, number of hydrogen bonds formed, ligand efficiency, intermolecular energy, torsional energy between the ligands and the drugs were recorded (Table 4). Calculations of binding energy, ligand efficiency, inhibition constant, intermolecular energy, Van Der Waals energy, electrostatic energy and ligand internal energy were generated with the AutoDock program as described in the Materials and Methods. The 10 conformations within each run were ordered based on binding energy. The conformation with the lowest energy was confirmed for the selection of the best compound.

Table 1. 2D and 3D structures with the molecular formula of the selected bio-active compounds from *Azadirachta indica*

Compound Name	2D	3D	Mol. Formula
Imidazole-2-carboxylic acid, 4-methyl			$C_5H_6N_2O_2$
Bis(2-propylpentyl) phthalate.			$C_{24}H_{38}O_4$
Dehydrodiisoeugenol			$C_{20}H_{22}O_4$
4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine			$C_{16}H_{14}N_2O_4$
Methylethyl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-5,6,7-trihydroindole-2-carboxylate.			$C_{21}H_{25}NO_4$

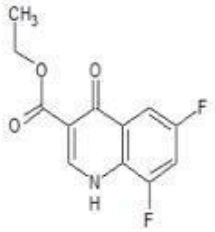
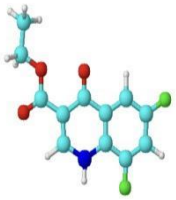
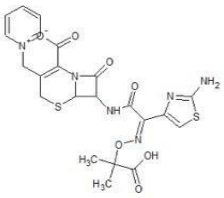
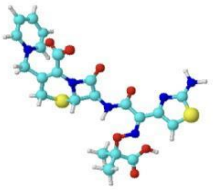
Compound Name	2D	3D	Mol. Formula
Ethyl 6,8-difluoro-4-hydroxyquinoline-3-carboxylate			$C_{12}H_9F_2NO_3$
Ceftazidime			$C_{22}H_{22}N_6O_7S_2$

Table 2. The table depicts the drug likeness properties calculation using MOLINSPIRATION

Compounds	M.wt	Hydrogen Bond Donor	Hydrogen Bond Acceptor	miLogP	Rotatable bonds	N-Violations	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-propylpentyl) phthalate	390.56	0	4	8.04	16	1	52.61	407.90	28
Dehydrodiisoeugenol	326.39	1	4	4.10	4	0	47.93	306.90	24
4-Dehydroxy-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-trihydroindole-2-carboxylate	355.43	1	5	4.54	6	0	68.40	335.84	26
Ethyl 6,8-	253.2	1	4	0.10	3	0	59.17	203.2	18

Compounds	M.wt	Hydrogen Bond Donor	Hydrogen Bond Acceptor	miLogP	Rotatable bonds	N-Violations	TPSA (Å)	Volume	N atoms
difluoro-4-hydroxyquinoline-3-carboxylate	0							0	
Ceftazidime	546.59	4	13	–5.68	9	2	191.23	439.78	37

Table 3. Binding energies between ompA protein and bio-active compounds from *A. indica*

OMPA 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	-5.88	-0.65	-6.47	-4.27	-2.2	0.6	-0.47
Bis(2-propylpentyl) phthalate	1	-3.69	-0.13	-8.46	-8.4	-0.06	4.77	-2.53
Dehydrodiisoeugenol	5	-5.88	-0.25	-7.37	-7.25	-0.12	1.49	-1.18
4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	3	-6.58	-0.3	-8.07	-6.81	-1.27	1.49	-0.45
Methylethyl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-5,6,7-trihydroindole-2-carboxylate	2	-5.31	-0.2	-7.1	-7.03	-0.08	1.79	-1.15
Ethyl 6,8-difluoro-4-	3	-5.31	-0.3	-6.2	-5.93	-0.27	0.89	-0.17

OMPA docking with compounds	Number of hydrogen bonds	Binding energy	Ligand efficiency	Intermolecular energy	vdW + Hbond+ desolv Energy	Electrostatic energy	Torsional energy	Total internal Unbound
hydroxyquinoline-3-carboxylate								
Ceftazidime	6	-6.94	-0.19	-10.22	-7.2	-3.02	3.28	-2.3

Table 4. Overall interactions of all the selected compounds with ompA of *A.baumannii*

PTK docking with compounds	Hydrogen bonds interactions	van der Waals interactions	π-σ interactions/ π-π T-shaped interactions/ amide-πstacked interactions	alkyl/π-alkyl interactions	Other interactions
Imidazole-2-carboxylic acid, 4-methyl-	ARG325 (2) ARG329 LYS322 (2) THR326	THR321 ALA326	-	-	LYS322 ARG329 (π- cation)
Bis(2-propylpentyl) phthalate	GLY309	PHE310 SER283 GLU280 GLN308 THR307	ALA311 LYS277 ARG281 LEU284(2)	-	GLY309 (Pi-lone pair)
Dehydrodiisoeugenol	ARG265 THR334 (3) ALA333	GLU229 ARG330 ILE316 GLN314 ALA333	HIS269 PHE332	-	-
4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	ASN227 LYS255 (2)	TYR259 ASP225 GLU229	LEU226 MET228 LYS251 VAL252	-	GLU248(π-anion)
Methylethyl 6-(4-ethoxyphenyl)-3-methyl-4-oxo-5,6,7-trihydroindole-2-carboxylate	ASP313 GLN314	SER283 ALA311 GLY309 GLN308	ARG281 PHE310	-	GLU280 (π- anion)
Ethyl 6,8-difluoro-4-hydroxyquinoline-3-carboxylate	ASP301 ARG304 (2)	ASN299 SER257 GLU254	VAL300 (2) ALA253 PRO260 LEU256	-	TYR298 (Pi-lone pair) ALA253(halogen)
Ceftazidime	ARG281 (3)	-	ALA285	-	-

PTK docking with compounds	Hydrogen bonds interactions	van der Waals interactions	π-σ interactions/ π-π T-shaped interactions/ amide-πstacked interactions	alkyl/π-alkyl interactions	Other interactions
	LYS238 SER239 ASN240		LEU278 ARG281		

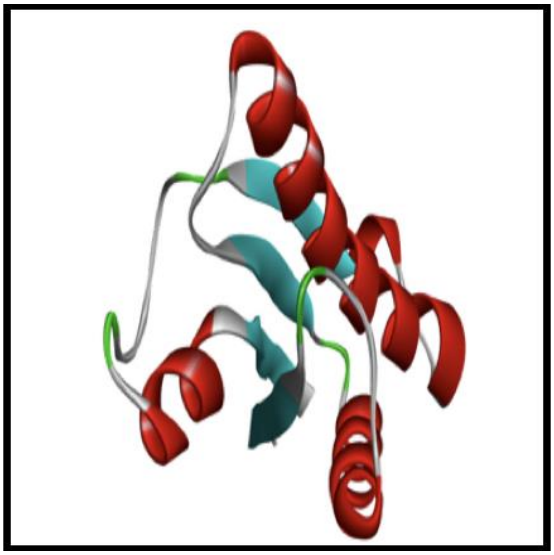


Fig. 1. 3D Structure of OMPA visualization using Biovia Discovery studio visualizer

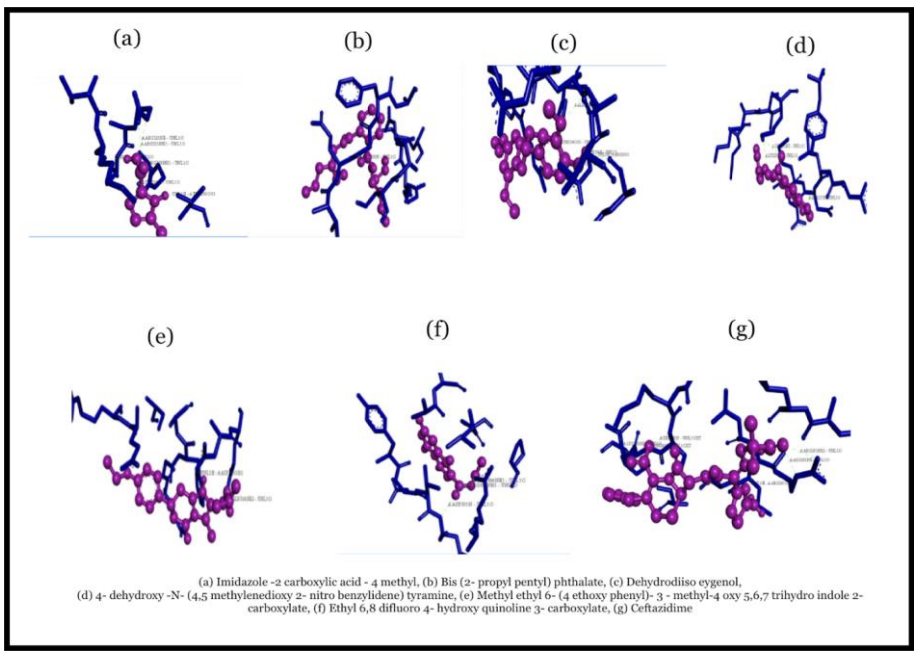


Fig. 2. Drug ligand docked interactions between the *A.indica* bio-compounds with ompA of *A.baumannii*

4. DISCUSSION

Acinetobacter baumannii is an arising nosocomial microorganism causing serious complications because of the inclination of its multi-drug safe property. Motility in *A. baumannii* may be because of the discharge of exopolysaccharide, making a film of high-molecular-weight sugar binding behind the bacterium to initiate and progress infections [22]. The intense virulence factor of *A. baumannii* is its capacity to form biofilms as a four significant step process viz., attachment of bacteria to the surface, development of micro colony, development of biofilms and finally its separation prompting further colonization. In *A. baumannii*, development of biofilm is intervened by cell to cell attachment through curli strands, attributing the virulence and pathogenicity [23]. Thus the present study is intended to target ompA protein of *Acinetobacter baumannii* with the bio active compounds from *Azadirachta indica* an in-silico approach. The systems of antimicrobial obstruction in *A. baumannii* strains of various origin, with the aim to consolidate or adjust the therapeutic treatment scheme utilized in the control of this nosocomial bacteria or execute cleaning and sanitizing systems to improve medical conditions. Curli mediate host cell adhesion and intrusion, and they are powerful inducers of the host inflammatory activity [24]. The design and biogenesis of curli are extraordinary among bacterial filaments that have been depicted to date. Primarily and biochemically, curli have a place with a developing class of filaments known as amyloids. Earlier studies demonstrate the occurrence of genotypic detection of putative virulence factors like ompA from *A. baumannii* to be 63.63%. The rise of extended range cephalosporin-safe gram-negative bacilli (RGN) is in effect progressively perceived. Colonization with RGN can occur endogenously through the development of ceftazidime resistance in previously susceptible gram-negative bacilli or exogenously through the cross-transmission of microorganisms between patients, the climate, or potentially health care workers [25].

The most prevalent gene was csuE (100%), followed by pgaB (98%), epsA and ptk (95%), bfmS (92%) and ompA (81%) among Virulence characteristics of multidrug resistant biofilm forming *Acinetobacter baumannii* isolated from intensive care unit patients. The alternative techniques to formulate botanical extracts of *Azadirachta indica* (neem) to improve its biological strength. In addition, it features both the significance of the arrangement of herbal items, which ought to be formulated with reproductive degrees of active compounds, and furthermore ought to be portrayed by utilizing logical instruments in quality control programs. Thus, it showed higher stability when contrasted with commercial items [26]. We also evaluated the impact of *A. indica* bio-compounds in the current investigation as many previous reports had detailed the characteristics of phenolic compounds up to its primary elucidations. Comparing the molecular weight of all the compounds, the least molecular weight of 126.11 was possessed by imidazole-2-carboxylic acid and the higher molecular weight of 390.36 was possessed by bis (2-propyl pentyl) phthalate ester. Other compounds showed a molecular weight ranging between 250 and 360. Evaluations on the hydrogen bonds donor and acceptor property, the greater number of rotatable bonds of around 16 were bis (2-propyl pentyl) phthalate ester along with the greatest miLogP estimation of 0.17. The TPSA value (Topological Polar Surface Area) of a compound is a significant assessment, as it attributes to the oral bioavailability of drugs which should be <140 Å. It is promising to take note that all the 6 bioactive compounds that we have selected showed TPSA estimations of <140 Å.

Evaluation of the overall docking energies showed that the greatest number of hydrogen bonds for imidazole-2-carboxylic acid while least for bis (2-propyl pentyl) phthalate. 4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene) tyramine shows the least binding energy of -6.58 whereas bis (2- propyl pentyl) phthalate shows about -3.69. Highest binding energy, least will be the avidity. Electrostatic, Torsional energy and Ligand efficiency were found to be greater in bis(2-propyl pentyl) phthalate ester. Earlier studies have documented a similar pattern of evaluation using the computational approach with the bio-compounds from A.indica against csgA [27]. In correlation with that, the present study had documented the applicability of the in-silico docking evaluations in assessing the inhibitory effect of natural compounds against any virulent target. The limitations of the study is that it was conducted as an in-silico observational study. Thus the future prospects are set to evaluate the antimicrobial activity using the *in-vitro* and *in-vivo* study models. Our team has extensive knowledge and research experience that has translated into high quality publications [28–32] [33–37].

5. CONCLUSION

The present study had documented the importance of ompA gene among the biofilm producing *A. baumannii* strains which might be considered as a serious threat in health-care settings. In-silico anti-biofilm inhibitory activity was promising with imidazole-2-carboxylic acid exhibiting a great interaction using computational investigation. However, the study requires further experimental investigation for the design of novel medications from *A. indica* to battle the threat of biofilm development in drug resistant strains, for example, biofilm producing *A. baumannii.*

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. The authors are grateful for the support given by Saveetha Institute of Medical and Technical Sciences, Saveetha Dental College and Hospitals, Saveetha Dental College and RJS Travels and Tours.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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