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The propensity of selected Indian plant extracts for polyphenolics, antioxidant, and inhibition of *Pseudomonas aeruginosa* biofilms through type-3 secretion system

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ABSTRACT

Pseudomonas aeruginosa as a bacterium has been evolved depending on its habit and the habitat causing acute and chronic infections in humans and animals. The infection is further facilitated with its modified behavioral and survival strategies which can resist antibiotics and other potential inhibitors. The formation of biofilms, the related cell-to-cell signaling, and the special cascade of effector proteins produced by the secretion systems, in particular, the type-3 secretion system (T3SS) adds as armor to the infection cycle. In the search for alternatives as well as adjuvants to combat antibiotic resistance, numerous commonly available weeds cited in Ayurvedic literature are an important avenue for studies. In this current study, an attempt to explore the ability of twenty such weeds against biofilms of *P.aeruginosa* was made. To get better insights into the mechanism of anti-biofilm activity of the hydroethanolic extracts, both *in vitro* (antioxidants and antibiofilm microtitre plate method) and *in silico* (molecular docking) approaches were tapped. The results indicate that extracts of Atibala (*Abutilon indicum*)and its polyphenolics possess good inhibitory activity against *P.aeruginosa* biofilm. This could be further exploited toward newer drug design and development.

1. INTRODUCTION

Many emerging bacterial infectious diseases (EBIDs) are a significant burden on global economies and public health [1,2]. Around thirty percent of fatalities worldwide is mainly because of bacterial infections leading to human dismalness and mortality. Understanding the global temporal and spatial patterns of EBIDs becomes a priority for the current scientific fraternity. One such organism of immediate attention for surveillance and epidemiological research is *Pseudomonas aeruginosa*, a gram -ve, encapsulated, facultatively aerobic rod-shaped bacterium, which is one among the six ESKAPE pathogens with a growing rate of virulence in humans and animals [3,4]. However, *P. aeruginosa* has evolved newer escape mechanisms to bypass chemical antibacterial agents bacteriostatic and/or bacteriocide [5,6]. This armored bacterial pathogen has developed resistance due to its switching abilities in physicochemical growth patterns of its niche between planktonic (free-floating) and biofilm (sessile) forms [7]. The biofilm development in pseudomonas involves a series of well-orchestrated steps starting from locating an adhesive surface, reversible adsorption on the substratum, production of exopolysaccharides, or exopolymeric substances (EPS), maturation, detachment, and co-infection [8-10].

During the biofilm stage an elemental phenomenon that occurs in bacteria, especially in *P. aeruginosa,* is quorum sensing that is cell-to-cell, density-dependent community cross-talk. It is observed that quorum sensing is responsible to facilitate thiol-disulfide redox metabolism of the bacterium [11-13]. Pseudomonas are prone to become resistant to antibiotics due to the formation of biofilms that complicates any treatment or infections. Further, 4-quinolones and N-acyl-homoserine lactones are involved as the major quorumone in signaling cascades [14,15]. Further, The main essential function of the prokaryotic cell is to transport proteins to the environment, compartments of cells, and other bacteria cells in pure culture or co-culture and mixed culture populations [16]. This mechanism is executed by an effector protein-mediated cascade known as the protein secretion system. There are different types of secretion systems, including Type 1 Secretion System, (T1SS), T2SS, T3SS, T4SS, T5SS, T6SS, and T7SS. In *P. aeruginosa* the T3SS, is mainly involved in virulence triggering oxidative stress (by generating reactive oxygen/nitrogen species) and could be majorly targeted to block bacterial signal

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trafficking. The bacterial flagellum and the injectisome are composed of conserved machinery known as the type III secretion system. This T3SS is the primary virulence determinant of *P. aeruginosa* that promotes tissue destruction and escapes phagocytosis by secreting numerous toxins that are translocated into host cells [17,18]. Type-3 cytotoxins play a crucial role during *P. aeruginosa* quorum-signaling, very importantly bi-functional cytotoxins ExoS and ExoT, along with ExoU have a cascade effect [19]. Electrostatic bonding determines the domain-substrate (ADP-ribosyltransferase) specificity in exo-cytotoxin protein modeling [20]. Henceforth, computational biology is employed to have a better insight into the complex fitting pieces of the puzzle. Tactically targeting *P. aeruginosa* T3SS-Exo system could be one of the possible ways to combat this pathogen.

With this background, it has been a priority to develop alternative approaches either preventive or therapeutic. Various methods are prescribed in classical, traditional plant-based Indian medicine for the development of effective drugs with lesser side effects but lack drug-target-based mechanistic design. The current study attempts to employ plant extracts from Indian herbs to effectively manipulate and percolate the thick EPS barrier ultimately targeting the bacterial strength or polysaccharide-mediated cell signaling. The details of the plants under investigation are listed in Table 1. A thorough literature meta-data analysis of the phytochemicals present in 20 selected plants revealed the presence of phyto-antibiofilm contributors such as quercetin, catechin, allicin, and eugenol which has previously been studied for antibacterial/antibiofilm activity [21-24]. However, the interaction of these phytochemicals was not evaluated against T3SS Exo-cytotoxin cascade. With this rationale, the phytomolecules were screened using *In silico* docking for their effective binding against T3SS targets (ExoS, ExoT, and ExoU) with corresponding binding energy *i.e.,* Atomic Contact Energy (ACE) or Gliding Energy indicating their inhibition [25]. Polyphenolics, the class of secondary metabolites are proven to exhibit antistress and antibacterial activity against pseudomonas [5] and profiling was evaluated along with various free-radical scavenging activities. Further, an *in vitro* experimental microtitre plate anti-adhesive model was set up to assess the anti-biofilm activity of selected twenty plant extracts (hydroethanolic extract 70:30 v/v). *In silico* modeling was also performed to understand the molecular-level interactions of the potent phyto-principles against T3SS cascade. The results of the current study infer to identify herbaceuticals that can eradicate *P. aeruginosa* biofilms ultimately leading to better health and hygiene.

2. MATERIALS AND METHODS

The chemicals, solvents, and reagents considered for the study were procured from Himedia Pvt. Ltd, Mumbai, India and were of extra pure analytical grade.

2.1. Plant Material

The medicinal plants considered for the current study are listed in Table 1. The leaves were collected with a time duration between March and July 2018 from Mysuru, Karnataka, India. For authentication on identification, the specimens were prepared and are deposited at the herbarium, Department of Studies in Botany, University of Mysore, Karnataka, India. The leaves of *Clerodendrum phlomidis* (Agnimantha), *Abutilon indicum* (Atibala), *Sida cordifolia* (Bala), *Coccinia grandis* (Bimboshta), *Oxalis corniculate* (Changeri), *Lantana camara* (Chaturangi), *Plumbago zeylanica* (Chitraka), *Acalypha indica* (Haritamanjari), *Martynia annua* (Kakanasa), *Solanum xanthocarpum* (Kanthakari), *Mimosa pudica* (Lajjalu), *Centella asiatica* (Mandookaparni), *Cyperus rotundus* (Musta), *Fumaria parviflora* (Parpata), *Cocculus hirsutus* (Patalagarudi), *Boerhavia diffusa* (Punarnava), *Osmium americanum* (Tulasi), *Zanthoxylum acanthopodium* (Tumbura), *Vetiveria zizanoides* (Ushira) and *Trachyspermum ammi* (Yavani) were cleaned and rinsed for surface sterilization with 0.5% sodium hypochlorite solution. All samples were air-dried under gentle sunlight for 48 h and the powder was stored until further use in zip-locked polythene containers.

Table 1: List of plants having potent antibiofilm activity on *Psuedomonas* species.

S. No.	Common name of the plant	Botanical name	Family	VSN
1.	Agnimantha	*Clerodendrum phlomidis*	*Verbinaceae*	VSN: 2135
2.	Atibala	*Abutilon indicum*	*Malvaceae*	VSN: 1454
3.	Bala	*Sida cordifolia*	*Malvaceae*	VSN: 1487
4.	Bimboshta	*Coccinia grandis*	*Cucurbitaceae*	VSN: 2157
5.	Changeri	*Oxalis corniculate*	*Oxalidaceae*	VSN: 1700
6.	Chaturangi	*Lantana camara*	*Verbinaceae*	VSN: 2144
7.	Chitraka	*Plumbago zeylanica*	*Plumbaginaceae*	VSN: 1721
8.	Haritamanjari	*Acalypha indica*	*Euphorbiacea*	VSN: 1070
9.	Kakanasa	*Martynia annua*	*Martyniaceae*	VSN: 1706
10.	Kanthakari	*Solanum xanthocarpum*	*Solanaceae*	VSN: 2058
11.	Lajjalu	*Mimosa pudica*	*Mimosaceae*	VSN: 1577
12.	Mandookaparni	*Centella asiatica*	*Apiaceae*	VSN: 673
13.	Musta	*Cyperus rotundus*	*Cyperaceae*	VSN: 242
14.	Parpata	*Fumaria parviflora*	*Fumiariaceae*	VSN: 1280
15.	Patala garudi	*Cocculus hirsutus*	*Menispermaceae*	VSN: 1540
16.	Punarnava	*Boerhavia diffusa*	*Nyctaginaceae*	VSN: 1648
17.	Tulasi	*Osmium americanum*	*Lamiaceae*	VSN: 132
18.	Tumbura	*Zanthoxylum acanthopodium*	*Rutaceae*	VSN: 1930
19.	Ushira	*Vetiveria zizanoides*	*Poaceae*	VSN: 508
20.	Yavani	*Trachyspermum ammi*	*Apiaceae*	VSN: 683

VSN: Voucher Specimen Number

2.1.1. For extraction

Briefly, around 25 g of the leaves from the selected 20 plants were individually mixed with 100 mL hydroethanolic solvent (70:30 v/v)

i.e., ethanol and water, respectively. Soxhlet apparatus was used with sample processer under 10 psi pressure using a vacuum flash evaporator [26,27]. The semi-solid residual photo-cocktail was filtered using a double-layered muslin cloth and centrifuged at 8000 rpm for 10 min. The supernatant extract was separated and was stored at 8°C until further.

2.2. Plant Extracts Profiling and Antioxidant Activity

Twenty hydroethanolic leaves extracts were subjected to total polyphenolic estimation. The free radical scavenging assay was assessed using 1,1-diphenyl-2picrylhydrazyl (DPPH), metal ion chelating activity using ferrozine, and reducing power activity with ferric chloride was performed respectively.

2.2.1. Estimation of total phenolic content (TPC)

The twenty leaves extracts were subjected to polyphenolic estimation using Folin-Ciocalteau method using gallic acid as standard (1 mg/mL) equivalents with 10 µL volume [26,28]. The optical density was read at 640 nm using a spectrophotometer. The TPC concentration was considered for all further quantitative estimation and bioactivity.

2.3. Evaluation of Radical Scavenging Activities of Selected Plant Extract

2.3.1. DPPH free radical scavenging assay

Quantitatively, the plant extract was subjected at 5 µg/mL, 10 µg/mL, 15 µg/mL, 20 µg/mL, and 25 µg/mL with gallic acid as a standard reference to assess the scavenging activity against DPPH free radical [26] and the optical density was read at 517 nm using a spectrophotometer. The percentage activity was calculated using the formula;

$$\text{DPPH scavenging activity } (\%) = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{üüü}}} \times 100$$ (Where A = absorbance)

2.3.2. Metal ion chelating assay

Gradient concentrations of plant extracts were evaluated for their metal ion chelating efficacy against ferrous iron-ferrozine complex, as previously described [26,28]. The optical density was read at 562 nm using a spectrophotometer. Ethylenediaminetetraacetic acid (EDTA) was considered as a standard reference and was calculated as per the above-mentioned equation.

2.3.3. Reducing power assay

All plant extracts were tested for their reducing potential using iron (III) as previously illustrated [26,29] and gallic acid (1 mg/mL) equivalents were used as standard. The optical density was read at 700 nm using a spectrophotometer.

2.4. Anti-biofilm activity

All twenty hydroethanolic crude extracts were subjected for assessment of the anti-biofilm activity of *P. aeruginosa* for their anti-adhesive, biofilm strength, and EPS inhibition using the microtitre plate method with crystal violet and ruthenium red staining as previously designed by our lab [7,8].

2.4.1. Biofilm production

The selected strain of *P. aeruginosa* was cultured in Luria-Bertani broth media with 0.6% yeast extract as nitrogen source and 0.5% glucose as an energy source under optimized parameters of pH and temperature as per Zameer *et al.* [5]. Samples were drawn at regular intervals and monitored for optical density to assess the growth curve of *P. aeruginosa*. Biofilm cultivation was carried out onto polystyrene 96 well microtitre plates. The plates were sterilized by the standard procedure described by Zameer *et al.* [7,8]. Further, 200 µL actively grown starter culture in microtitre wells were inoculated. The same was used to quantitate biofilm strength, EPS-using crystal violet, and ruthenium red staining respectively [30].

2.5. Quantification of Biofilm

2.5.1. Biofilm strength assay

The strength of biofilm was quantified as per the protocol described by Djordjevic *et al.* [31], with minor modification using the gradient concentrations of the plant extract inhibitors at 25 µg/mL. Briefly, the staining of adherent bacteria onto the cultivating surfaces was carried out using polystyrene 96 well microtitre plates. The surfaces were washed thrice with 250 µL of PBS (pH 7.4) during which non-adherent bacteria were washed out. The plates were dried in an inverted position at 42°C for 30 min. The growing biofilms were scraped using a sterile scalpel and were collected in sterile vials. Further, 150 µL of 1% crystal violet solution was added to each well and further incubated at 37°C for 30 min. Later, samples were carefully rinsed with water to decant the excess dye. 200 µL of 95% ethanol was used as dye solubilizer with subsequent incubation at 4°C for 45 min. Samples were drawn from individual wells and were monitored at 595 nm using a microtiter plate reader [7,8].

2.5.2. EPS inhibition assay

The production of EPS was carried out using the ruthenium red staining technique according to Borucki *et al.* [30], using the gradient concentrations of the plant extracts inhibitors at 25 µg/mL. Briefly, biofilms were stained for matrix EPS with the addition of 200 µL of 0.1 % ruthenium red to all the vials and wells which were incubated for 45 min at RT. Further, the media obtained from the wells was quantitated at 450 nm using a microtiter plate reader. The amount of dye reduced was compared and calculated with an average measurement of the blank [7,8] and the corresponding percentage inhibition was calculated.

2.6. Molecular Docking

Structure-activity relationship studies provide insight into the target-ligand interactions [32,33]. The proposed study considers three major effector proteins of T3SS in *P. aeruginosa* responsible for their role in pathogenesis and acute infections [34,35]. The crystal structure of the targets, namely, ExoS (Protein Data Bank [PDB]: 1HE9), ExoT (PDB: 6GNN), and ExoU (PDB: 4AKX) were retrieved from PDB. The ligand molecules were docked onto the active site of the receptors considering phytochemical evidence and abundance of the plant extracts from the previous studies in the selected plants. Allicin (PubChem: 65036), Catechin (PubChem: 9064), Eugenol (PubChem: 3314), and Quercitin (PubChem: 5280343) were taken into account depending on their various bioactivities. These ligands are currently been proven for their antibacterial properties. Molegro software package was used and the results were analyzed considering the ACE and gliding values [25,36]. The negative values indicate higher binding efficacy in the active site pockets between the ligand and the target indicating active inhibition [37].

2.7. Statistical Analysis

All data were processed for statistical validation using GraphPad Software, Inc., (version 6.0, California, USA). The experiments

were designed in triplicates and three independent experiments were performed. The results were expressed in mean ± standard deviation ($n = 3$) with standard error bars indicated in the graph. One-way analysis of variance, followed by Duncan's multiple range test was performed with $P < 0.05$. The percentage of inhibition for the phytoextracts was determined in terms of IC_{50} with regression correlation studies considering the polyphenolics.

3. RESULTS AND DISCUSSION

Microbes have co-evolved and have become an integral component of the host system and humans are no exception. This ubiquitous ecological interaction might vary in their role plays from being symbionts, mutualists, and antagonists. Microbes are fundamentally involved in the homeostasis of the metabolic, physiological, and immune systems regulations. However, these tiny-complex organisms have another detrimental phase as pathogens, and their interaction in the host-microbe association is not well understood, which could essentially affect the survival of the host leading to death. Recent advancements in drug design, newer antimicrobials, vaccine therapy, anti-viral therapy, anti-microbial peptide path-blockers, membrane disruptors, and host-defense reinforcement have made a significant impact in combating pseudomonas-related infections. Further, continuous implications of antibiotics have provoked the emergence of extensively drug-resistant, multidrug-resistant, extended-spectrum β-lactamase, and carbapenemase-producing pseudomonal strains which have imposed a serious therapeutic challenge. These events render even the most effective drugs ineffective and hence pose a threat to food, meat, and dairy industries extrapolating their infectious existence in humans causing cystic fibrosis, urinary tract, and nosocomial infections. Hence, understanding the phytomolecules in terms of their polyphenolics, antioxidant, and anti-biofilm potency becomes the prime focus of the current study.

3.1. Plant Extract Screened in the Current Study

The study aims to explore complementary and alternative medicine as a holistic approach to screen plants as anti-biofilm contributors for *P. aeruginosa*. Hence most of the plants considered for the study are the common weed [Table 1] found in Karnataka, India throughout the year without much seasonal variation and mentioned Indian traditional medicine for their curative function. In any disorder/infection, free radicals play a pivotal role in intensifying pathogenesis and T3SS is no exception. Henceforth, in the current study, we have attempted in exploring the photobiological for their antioxidant

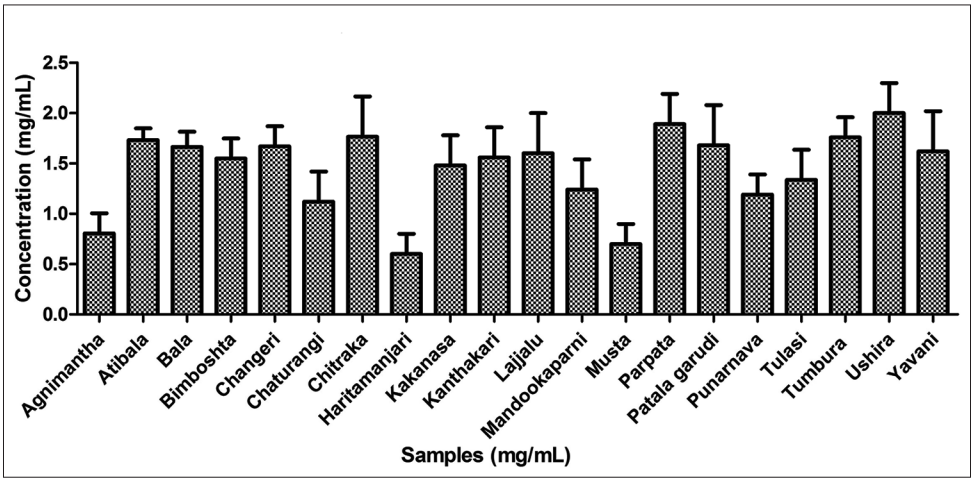

Figure 1: Total phenolic content of twenty hydroethanolic plant extracts.

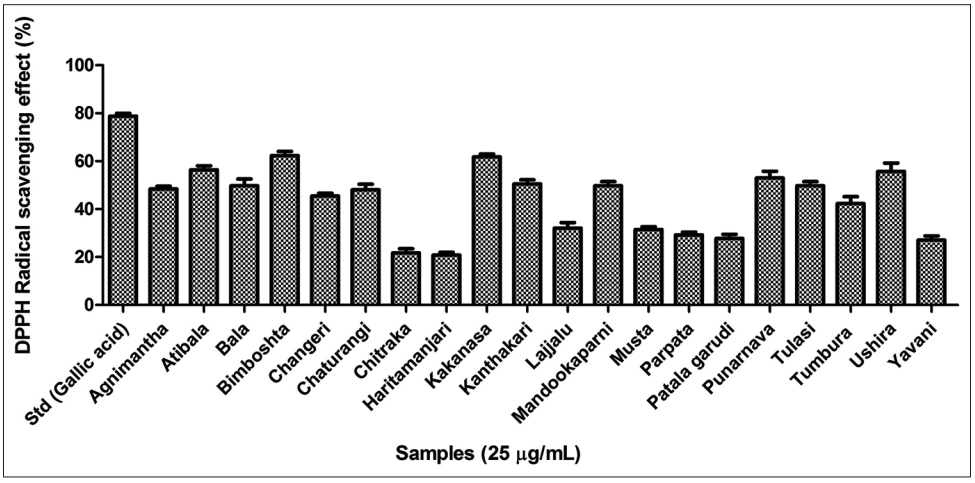

Figure 2: Antioxidant assay of twenty hydroethanolic plant extracts using DPPH free radical scavenging assay.

potency and antibiofilm efficiencies using hot hydroethanolic leaves extracts [38,39].

3.2. Evaluation of Polyphenolics

Polyphenols are the group of secondary metabolites which are produced by the plants as defensins to avoid infections from rhizosphere intruders. These include the wide class of alkaloids, flavonoids, tannins, saponins, and terpenoids at large. In this study, the twenty plant leaves extracts were estimated for their total phenolics for understanding the abundance of the total phenolic content (TPC) present in the plant leaves, and the same is further used as a quantitative measure in all estimations and assays. Among the plants screened Ushira, Parpata, and Chitraka were the top three plants with the highest TPC as depicted in Figure 1.

3.3. Assessment of Antioxidant Efficacy

Three major antioxidant scavenging assays were performed for all the twenty leaves extracts. The results of the DPPH free radical scavenging

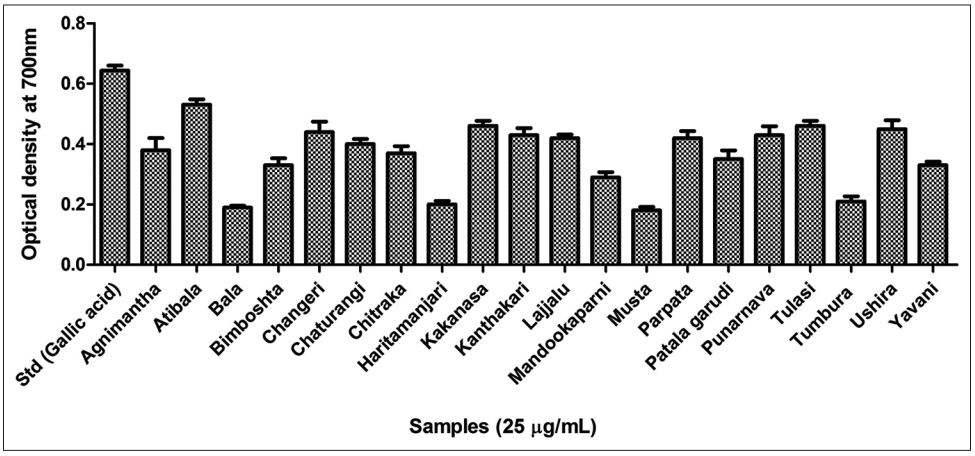

Figure 3: Antioxidant assay of twenty hydroethanolic extracts using metal ion chelating assay.

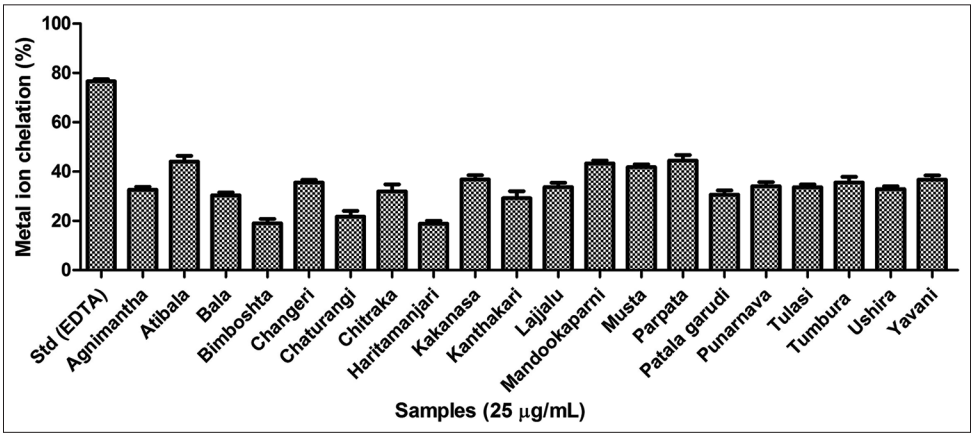

Figure 4: Antioxidant assay of twenty hydroethanolic extracts using reducing power assay.

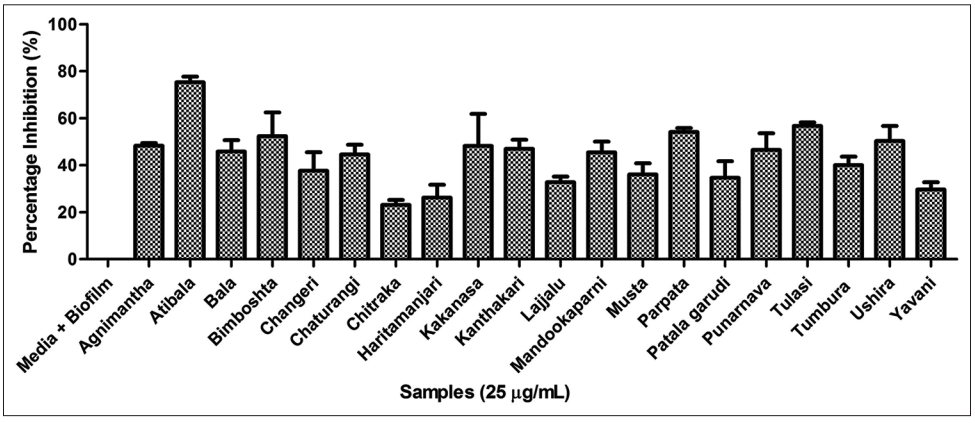

Figure 5: Assessment of biofilm strength using crystal violet method microtitre plate method.

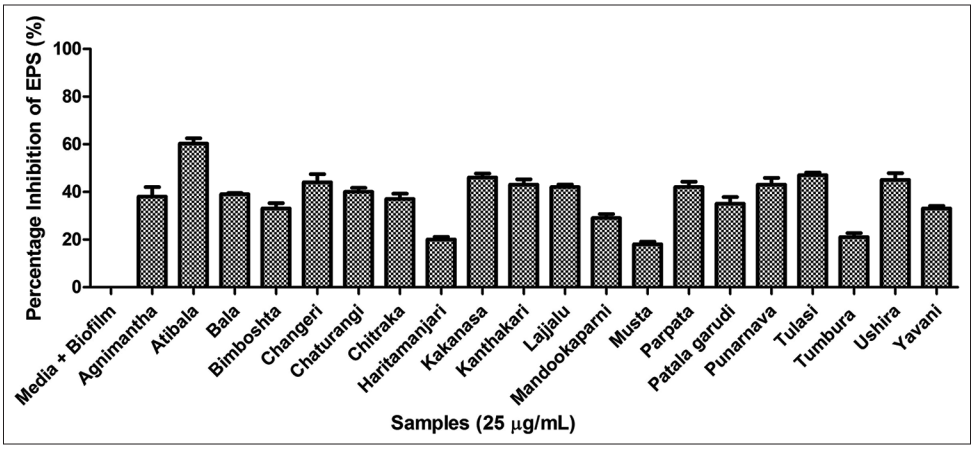

Figure 6: Assessment of EPS using ruthenium red method microtitre plate method. EPS: Exopolymeric substances.

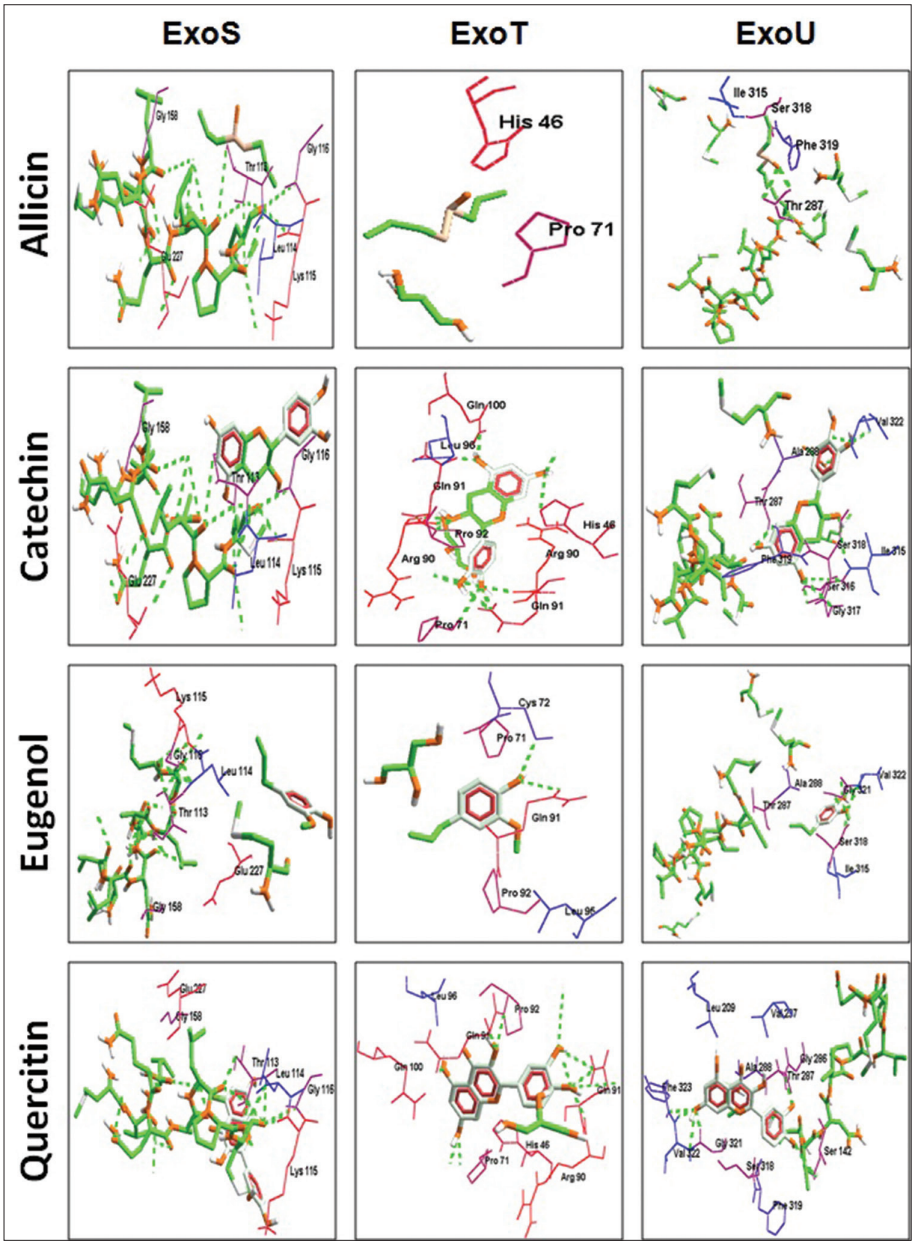

Figure 7: Molecular docking of the potent phyto-ligands with the target for understanding structure-activity relationship studies.

assay inferred the highest activity by Bimboshta, Kakanasa, and Atibala respectively as reflected in Figure 2 and compared to gallic acid as standard. Further, for the metal ion chelating efficiency Atibala, Parpata, and Mandookaparni exhibited better activity compared to EDTA as standard [Figure 3]. Further, reducing power potency Atibala, Tulasi and Ushira exhibited maximum activity as mentioned in Figure 4. The results of all three antioxidant assays reflect the ability of plant polyphenols to effectively function as antioxidant contributors [40].

3.4. Assessment of Antibiofilm Potency

The phenolics can function as anti-quorumone molecules against bacterial biofilms [41]. Henceforth, the plant extract was further analyzed for its efficacy to inhibit biofilm strength and EPS. The 96 well plate method was performed to analyze the anti-adhesive activity of *P. aeruginosa* biofilms. Among twenty extracts Atibala, Tulasi and Parpata exhibited maximum activity as reflected in Figure 5. The EPS was inhibited by Atibala, Tulasi, and Kakanasa extracts respectively [Figure 6].

3.5. Molecular Docking of the Phyto-attributes on the T3SS-Exo System

For better insights into the mode of action phytomolecules (ligands) under investigation namely allicin, catechin, eugenol, and quercitin were docked on the T3SS effector proteins ExoS, ExoT, and ExoUwhich were not previously studied against the selected ligands. The results are depicted in Figure 7 as docking poses and Table 2 illustrates the atomic contact energy values and gliding values [37]. The result indicated that quercetin and catechin could inhibit ExoS and the latter inhibits both ExoT and ExoU very effectively with the highest ACE values [42]. From Figure 8, it is very evident that these phytochemicals are effective against the oxidative species regulating pyocyanin, elastase, and superoxide dismutase (SOD1) as well as exotoxin A, ExoS, and ExoU, their effect is observed on pro-

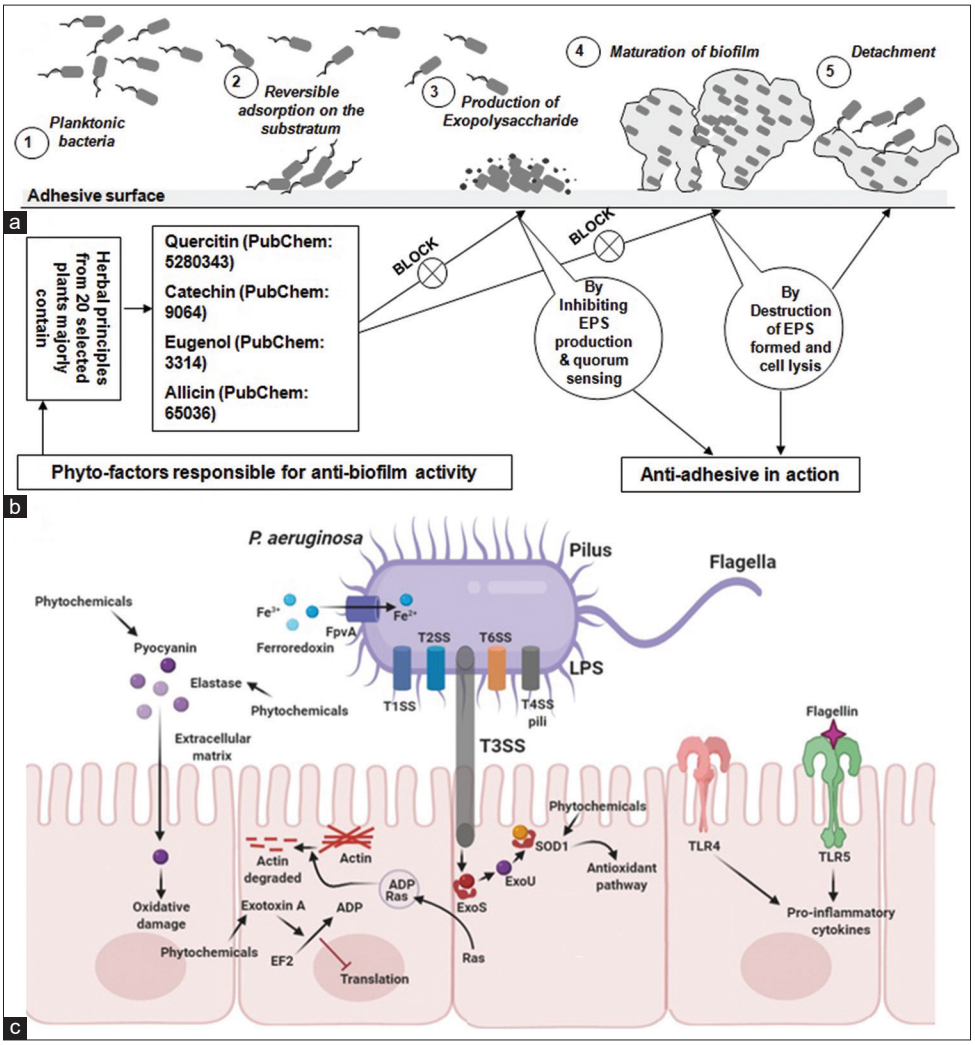

Figure 8: Overview of the probable mode of action. Panel A: Indicates the various stages of biofilm development in *Pseudomonas aeruginosa*. Panel B: Depicts the mode of action of phyto-contributors in inhibiting EPS and biofilm strength and lysis. Panel C: Cellular level interaction of phytochemicals with the potential targets of T3SS-Exo cytotoxic cascade. EPS: Exopolymeric substances, T3SS: Type-3 secretion system.

Table 2: Molecular docking and SAR of the four potent phytomolecules namely allicin, catechin, eugenol, and quercetin were docked with the target molecule of T3SS namely ExoS, ExoT and ExoU respectively.

Name of the biomarker	Name of the compound	Details of H-bond interaction			ACE values	Glide values in Mol/J	Amino acid residues of docked domains
		Number of bond	Bond length	Bond energy			
ExoS	Allicin	16	−2.5 −1.62 −1.85 −2.32 −2.5 −2.5 −2.5 −2.5	2.87 3.26 3.22 3.13 2.67 2.95 2.63 2.90	−181.65	−3.63	Thr 113, Leu 114, Lys 115, Gly 116, Gly 158, Glu 227
	Catechin	16	. −2.5 −1.62 −1.85 −2.32 −2.5 −2.5 −2.5 −2.5	2.87 3.26 3.22 3.13 2.67 2.95 2.63 2.90	−163.97	−3.27	Thr 113, Leu 114, Lys 115, Gly 116, Gly 158, Glu 227
	Eugenol	16	. −2.5 −1.62 −1.85 −2.32 −2.5 −2.5 −2.5 −2.5	−2.87 3.26 3.22 3.13 2.67 2.95 2.63 2.90	−132.37	−2.64	Thr 113, Leu 114, Lys 115, Gly 116, Gly 158, Glu 227
	Quercetin	16	−2.5 −1.62 −1.85 −2.32 −2.5 −2.5 −2.5 −2.5	2.87 3.26 3.22 3.13 2.67 2.95 2.63 2.90	−234.39	−4.68	Thr 113, Leu 114, Lys 115, Gly 116, Gly 158, Glu 227
ExoT	Allicin	–	–	–	−191.99	−3.83	His 46, Pro 71
	Catechin	14	−1.61 −2.27 −2.5 −2.5 −2.5 −2.5	3.27 3.14 2.90 3.07 3.06 2.96	−208.53	−4.17	His 46, Pro71, Arg 90, Gln 91, Arg 90, Gln 91, Pro 92, Leu 96, Gln 100
	Eugenol	2	−2.22	3.15	−165.23	−3.30	Pro 71, Cys 72, Gln 91, Pro 92, Leu 95
	Quercetin	11	−2.5 −2.5 −2.17 −1.96 −1.94 −2.5	3.02 2.96 3.16 3.20 3.21 3.05	−204.66	−4.09	His 46, Arg 90, Gln 91, Pro 71, Gln 91, Pro 92, Leu 96, Gln 100
ExoU	Allicin	2	−0.02 −0.28	3.58 3.51	−247.01	−4.94	Thr 287, Ile 315, Ser 318, Phe 319
	Catechin	8	−2.5	2.715	−309.64	−6.19	Thr 287, Ala 288, Ile 315, Ser 316, Gly 317, Ser 318, Phe 319, Val 322
	Eugenol	5	−2.5	2.92	−170.71	−3.41	Thr 287, Ala 288, Ile 315, Ser 318, Gly 321, Val 322
	Quercetin	6	−1.18 −0.03	3.36 3.42	−300.12	−6.00	Ser 142, Leu 209, Gly 286, Thr 287, Ala 288, Val 297, Ser 318, Phe 319, Gly 321, Val 322, Phe 323

SAR: structure-activity relationship, T3SS: Type-3 secretion system, ACE: Atomic contact energy

inflammatory cytokines (TLR4 and TLR5). These phytomolecules could be further explored for their nanoparticles to facilitate higher activity [34,43-45] and better percolation into the biofilm cluster facilitating insights into the mechanistic understanding of the host-pathogen interactions [46-49].

4. CONCLUSION

The results reflect the efficacy of the 20 selected plant extracts to exhibit potent antioxidant and antibiofilm activity against *P. aeruginosa*. Further studies have to be explored for having better insights into the characterization of the pure compounds and to understand the mechanistic action on the T3SS cascade at a cellular and molecular which could be efficient in developing newer drug targets against *P. aeruginosa* biofilms in specific and bacterial infections in general.

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6. AUTHORS CONTRIBUTION

Avinash MG carried out the experiments, assimilated data, and drafted the manuscript. Farhan Zameer designed the protocol and Shubha Gopal analyzed data and edited the manuscript.

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8. CONFLICT OF INTEREST

The authors express no conflict of interest.

9. ETHICAL APPROVALS

Not Applicable.

10. DATA AVAILABILITY

Not Applicable, raw data files could be provided upon request to the authors.

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