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In silico Herbal Bioprospection targeting Multi-drug resistant *Pseudomonas aeruginosa*

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Abstract

The re-emerging drug resistant strains of *Pseudomonas aeruginosa* require novel drug development strategies. A 3-step matrix based herbal bioprospection approach was devised to identify potential herbal leads targeting process of spread of micro-organism in the host cell. 05 bioactivity parameters were selected on the basis of extensive literature surge relevant to confer multi-drug resistance among *P. aeruginosa.* Biofilm formation and Quorum sensing exhibited maximum relevance as physiological targets. The binary matrix score analysis of database of 38 plants identified using classical bioprospection filtered 10 herbal exhibited potential to mitigate ≥3 virulence factors. The weightage matrix score analysis excluded 05 herbals having score less than median weightage matrix score i.e., 12.66. The optimization of bioassociational data on a scale of 0-1 using fuzzy score matrix analysis led to the selection of *Glycyrrhiza glabra* ***(Gg),*** *Terminalia chebula* ***(Tc),*** *Aloe barbadensis* ***(Ab)*** *and Mentha piperita* ***(Mp)*** as potent herbal leads with μS > 0.5 (~median fuzzy score) and *Zingiber officinale* ***(Zo)*** selected as control lead molecule. These herbal leads will be subjected to *in vitro* analysis in future studies.

Keywords: *Pseudomonas aeruginosa*, Multi-drug resistance, 3-step matrix based herbal bioprospection; herbal informatics

Introduction

There are > 3 lakh medicinal plants known from ancient literature that have been extensively employed to cure various maladies.[1] The evidence of such alternative/complement medicine through stages of (a)"Doctorine of Signature" era; (b)time-dependent trial and error/validation era; (c)experience driven protocols era to present day classical and herbal holistic approaches.[1] However, the lack of standardization of validated protocols and limited methodologies of 'process authentication' & 'preparation' has evolved a non-catalogued herbal literature limiting deciphering its potency.

The compartmentalized evolution of micro-organism though parallel to herbals, converges with human evolution being symbiotic in nature. The industrial upsurge and increase in global population in last century has constrained these barriers of speciation, in turn, exhibited a rapid rise of drug-resistant micro-fauna. *'Pseudomonas aeruginosa'* is another such multi-drug resistant gram-negative bacterial species generally spreading through nosocomial i.e. hospital-acquired infections. *P. aeruginosa* is indicted in eye, ear, chronic respiratory, hospital-acquired pneumonia, chronic intra-abdominal, urinary tract, skin and soft-tissue infections etc. [2, 3] The nutritional versatility and its ability to grow in various natural habitats make it a pertinent threat for humans, eg. 55% average precedence rate in Indian sub-continent. [4, 5] It is the second most common reason of nosocomial pneumonia (18.1%),

third most frequent pathogen causing urinary tract infection (16.3%) and eighth most common isolated pathogen from blood stream (3.4%).[6] High morbidity and mortality rate associated with nosocomial pneumonia has reached 20-70% especially those having compromised natural defense eg. Cystic fibrosis patient. [7-10] As per CDC (USA) report of 2013, >50,000 annual infections led 400 deaths among 6000 confirmed diagnosed victims exhibited its severity. However, the data of developing countries might differ extremely among various countries. Combination of anti-pseudomonal drugs like Colistin, β-lactams (Penicillin, Piperacillin); Cephalosporin (Ceftazidime and Cefipime); Aminoglycosides (Gentamycin and Clindamycin); Carbapenem (Imipenem and Meropenem) and Quinolones (Ciprofloxacin) are being widely used to manage such infections. The antibiotic-resistant in *P. aeruginosa* could be attributed towards selective pressure of antibiotic drugs, evolution of microflora, increase in vulnerable population profile and failure of treatment modalities at hospital level.[11] As it is very familiar that traditional system of medicine based on plants can play an important role in providing health care. Ayurveda, Unani, Siddha, Naturopathy and Homeopathy these are the basic recognized systems of medicine in India. Plants have formed the foundation of these therapeutic systems.[12] Thus, there is a need to investigate new antimicrobial drugs utilizing herbal informatics approach against this continuously evolving pathogen.

In the present study, herbal informatics model was used to catalogue promising medicinal plants by utilizing integrated biodynamic, bioprospection and biostatistical scoring approach.[13] This model includes identification of virulent factors/bioactivity parameters based on their classical bioprospection (using literature survey) and cataloguing or categorizing them with respect to pathophysiology of targeted pathogen (*P. aeruginosa*). The present study is aimed towards identification of potent herbal leads in mitigating drug resistance in *P. aeruginosa.*

Materials and Methods

Identification of bioactivity parameters/ physiological targets

A number of significant bioactivities were analyzed using literature based analysis of mechanistic aspects. Five testing parameters were found to be relevant for the present study. These include: (a) Biofilm formation inhibition; (b) Quorum Sensing inhibition; (c) Type III secretion system inhibition; (d) Exoenzyme S inhibition; (e) Exotoxin A inhibition. [14-19]

Evaluation of priority index for bioactivities

This step is achieved by determining the evaluation of relevance for each bioactivity parameter, based on scoring matrix approach with the help of PubMed, utilizing Academic Search Engine Optimization (ASEO) search platform. [20] Advanced Search was carried out with query set and is cited under quotes (‘ ’), for example:- ‘Bioactivity parameter + Antimicrobial activity’. This yields ‘N’ no. of total hits against a query cited. The first prioritized (n=20) sample searches were screened subjected to an individual analysis of each factor. On the basis of Coefficient of association the relevant hits were determined followed by calculating the percentage relevance by using the formula given below:

$$\text{Percentage Relevance} = \frac{\text{Relevant Hits}}{20} \times 100$$

Identification of promising leads based on classical bioprospection

On the basis of following descriptors a list of herbals was prepared with the help of extensive literature surge (classical bioprospection): (a) Ethnopharmacological importance of plant; (b) Relevance of Herb in traditional medicine; (c) Availability

factor in localized regions; (d) Any vedic literature supporting its use and; (e) Prior investigations on potential of the herb to the disease.

Binary matrix based selection

Coefficient of association with each bioactivity parameter is the decisive factor to determine plant's binary matrix that is based on the presence and absence of particular inhibiting property exhibited against individual physiological target. [21] The score lies between 0 to 5, while the cut-off value is the median i.e., 3. Plants having score ≥ median value were considered for weightage based matrix analysis.

Screening of plants on the basis of total binary weightage scores

This step differentiates plants with high combined weighed score, determined by addition of weightage of bioactivity parameters present in a particular plant species.

Fuzzy concept to filter out promising plants

The working principle of this step is fuzzy logic or many- valued logic. These values were determined by using the formula given below, in order to make the score of selected plants in a range between 0 to 1. The plants with the obtained value 1 are considered to be surely effective while those with value 0 are neglected and plants with value lesser than 1 but greater than 0 are considered to be partly effective. [22, 23]

$$\mu s = \frac{[(S) - \min(S)]}{\max(S) - \min(S)}$$

where, μS is the Fuzzy value; S is the Weightage matrix score.

Optimization of data using decision matrix score

In this approach the numerical value of scores obtained were converted into a leveled score by using a scaled magnitude represented by a symbol. This is to present obtained data into an optimized symbolic way. [24] The optimized data analysis revealed the leads that should be considered for consequent step.

Statistical analysis

The data based analysis was conducted thrice to assess time based variation in a stipulated period. The significance of tested variables was evaluated by using comparing mean values. Confidence level chosen for the study is $P < 0.05$.

Results

In the present study the precedence of bioactivity parameters was evaluated on the basis of comparative % relevance scores obtained by weighted matrix score based analysis (Table-1). The order of relevance was found to be: Biofilm formation> Quorum sensing > Exotoxin A >Type III secretion system > Exoenzyme S (Fig 1). [17, 25-31]

The 38 plants were identified on the basis of their comparative ethnopharmacological importance, relevance of herb in traditional medicine, their availability factor in localized regions, any Vedic literature supporting their use and prior medicinal investigation (Table 2). [21-23, 32-42]

Binary (presence-absence) coefficients matrix based analysis of identified plants (~38) revealed that, 10 plants namely, *Glycyrrhiza glabra* ***(Gg)***, *Terminalia chebula* ***(Tc)***, *Aloe barbadensis* ***(Ab)***, *Mentha piperita* ***(Mp)***, *Foeniculum vulgare* ***(Fv)***, *Zingiber officinale* ***(Zo)***, *Barberine vulgaris* ***(Bv)***, *Azadiracta indica* ***(Ai)***, *Matricaria recuitata* ***(Mr)*** *and Bacopa monnieri* ***(Bm)*** exhibited activity against ≥3 virulent factors as represented in Table 3.

The herbal leads(~10) selected in previous step were subjected to weighted matrix score analysis utilizing weighted relevance of each bioactivity under consideration (Table-1). Such analysis revealed 05 potent herbal leads with weighted matrix score ≥ 12.66 (Fig 2). Fuzzy set matrix based optimization of top 10 herbals utilizing relative priority scale of 0-1 led to exclusion of *Matricaria recuitata* ***(Mr)*** *and Bacopa monnieri* ***(Bm)*** with $\mu S < 0.1$ (Table 4). The final analysis revealed that Glycyrrhiza *glabra* ***(Gg),*** *Terminalia chebula* ***(Tc),*** *Aloe barbadensis* ***(Ab)*** *and Mentha piperita* ***(Mp)*** as leads with $\mu S > 0.5$ (median fuzzy score) and *Zingiber officinale* ***(Zo)*** selected as control lead molecule.

Discussion

Emerging and re-emerging invincible drug resistant strains warrants for rapid investigation of effective antimicrobial modalities.[43, 44] The exploration of utility of ethnopharmacological importance of medicinal plants is always a research quest to resolve this challenging issue of multi-drug resistance. The present study demonstrated *in silico* approach to target numerous bioactivities like Exotoxin A; Type III secretion system and Exoenzyme S of *P. aeruginosa* responsible for contribution towards emergence of multi-drug resistance. Exotoxin A blocks protein synthesis by ADP ribosylation of elongation factor 2, in that way causes cell death. [16] Type III secretion system is a significant virulent factor that is responsible for transferring effector toxins into the host cell. [17] Exoenzyme S has been reported to block fluid-phase endocytosis, hinder intracellular trafficking thus inhibits phagocytosis of bacterium.[14]

The fuzzy score based optimization revealed that *Glyccyrhiza glabra (Gg); Terminalia chebula (Tc); Aloe barbadensis (Ab); Mentha piperita (Mp); Foeniculum vulgare (Fv); Berberis vulgaris (Bv)* and; *Azadiracta indica (Ai)* are potent herbal leads against *P. aeruginosa.* These herbal leads were selected on the basis of binary matrix (i.e. present or absence of the bioactivity) is in line with work reported by Cheetham and coworkers (1969) that made use of binary coefficients for interpretation of multivariate bioassociational data.[21] One of the major process of inhibition of resistance offered by *P. aeruginosa* are anti-biofilm formation via disruption of Quorum sensing pathways essential for colonization and growth of micro-organisms.

Inhibition of Bioflim activity & Quorum sensing pathways

Biofilm, a protective shield encapsulating the micro-organism, prevents penetration of antibiotics[19]. The phenolics -Ellagic acid (***Tc***), chebulic acid (***Tc***) & gallic acid (***Tc***)[45]*;* glycoside - Rutin (***Ab***), phenyl propene - Eugenol *(**Ab**)* & terpene - Limonene (***Ab***)[46]; essential oils - Limonene, α-pinene & β-pinene (***Mp***)[45,46] and; gingerol (***Zo***)[47] were reported to inhibit biofilm formation. The phenolic compounds of *Terminalia chebula* down regulate the gene expression of Las and Rhl system of quorum sensing of *P. aeruginosa*.[48] The anti-quorum sensing activity of Gingerol (***Zo***), Shogaol (***Zo***) and Azagingerol (***Zo***) was reported to be attributed to their long alkyl chains, having structural similarity to N-acyl homoserine lactone. Such competitive binding to transcription

regulator LasR reduces the expression of virulence factors like elatase, protease, exotoxin A and LasI.[47] These identified indicators can be utilized to screen 'drug like molecules' from these prioritized herbal leads.

Conclusion

Our analyses have demonstrated 3-step matrix based herbal informatics approach,a fast, reliable and systematic model to target multiple pathophysiological foci altered during onset and spread of disease. Such model works on the principle of multivariate analysis of bioassociational data with segregation and filtration at each stage. This study has provided 05 herbal leads against *P. aeruginosa* which, in turn, needs to be evaluated further at *in vitro* and *in vivo* level.

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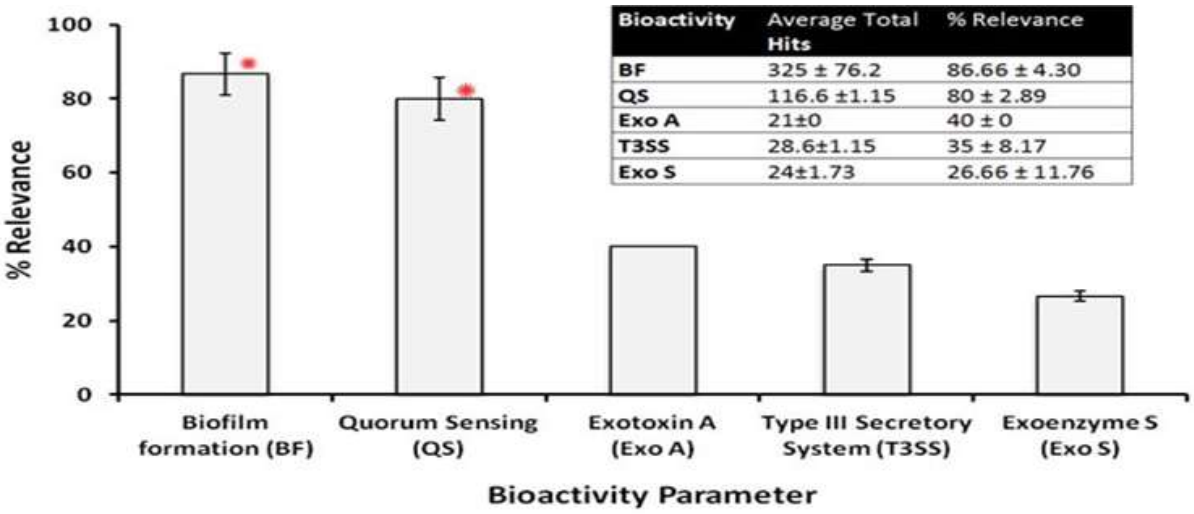

Bioactivity	Average Total Hits	% Relevance
BF	325 ± 76.2	86.66 ± 4.30
QS	116.6 ±1.15	80 ± 2.89
Exo A	21±0	40 ± 0
T3SS	28.6±1.15	35 ± 8.17
Exo S	24±1.73	26.66 ± 11.76

Fig 1: Percentage Relevance of Identified Bioactivity Parameters using Scoring Matrix based analysis

*Indicates BAP exhibited significantly highest % relevance ($p<0.05$) as compared to other parameters i.e., Pili, fimbrae, type II secretory system etc.% Relevance was calculated using the formula:

$$\% \, Relevance = \frac{No.\,of\ relevant\ hits}{n = 20} \times 100$$

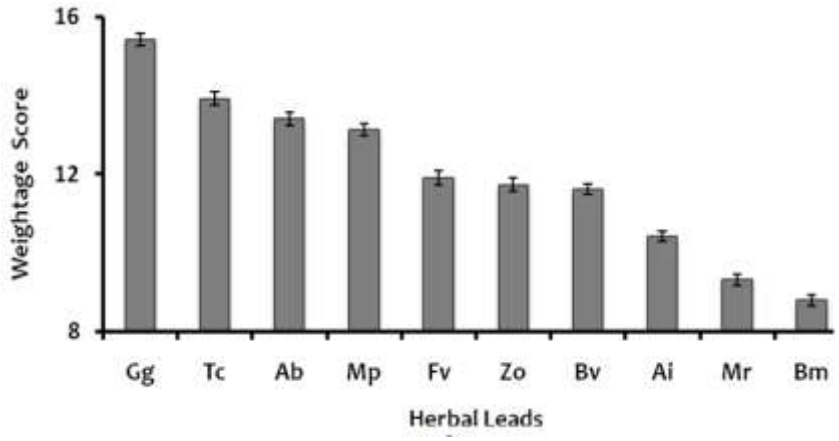

Fig 2: Weightage matrix scores for herbals

Weighted Matrix scores of herbal leads identified using Binary matrix analysis with respect to BAP, Gg (*Glycyrrhiza glabra*) = 15.42; Tc (*Terminalia chebula*) = 13.92; Ab (*Aloe barbadensis*) = 13.40; Mp (*Mentha piperita*) = 13.32; Fv (*Foeniculum vulgare*) = 11.90; Zo (*Zingiber officinale*) =11.73; Bv (*Berberis vulgare*) =11.62; Ai (*Azadiracta indica*) = 10.42; Mr (*Matricaria recuitita*) = 9.32; Bm (*Bacopa monnieri*) = 8.8.

Table 1: Rationale for the selection of Bioactivity parameters.

S. No.	Bioactivity Parameter	Role
1.	Biofilm	i) Biofilm is made up of extrapolymeric substance (EPS) that involves polysaccharide(Psl, Pel and Alginate), proteins(Type IV pili, Flagella, Fimbriae and CdrA [cyclic-di-GMP-regulated Adhesin]) and eDNA(extrcellular DNA) as functional moieties that enhances resistance towards action of antibiotics. ii) The process involves reduced penetration into the bacterial cell by tobramycin, aminoglycosides and colistin due increased thickness of biofilm and modification in outer membrane. iii) Plants like *Warburgia salutaris* and *Zingiber officinale* etc., have been reported as potent inhibitors of Biofilm formation.
2.	Quorum sensing(QS)	i) QS is an intercellular communication phenomenon, also regulate the expression of numerous genes by regulatory signals by binding to the promoter region of gene of the virulence factor. ii) It mainly involves two system Las (Las I and LasII) and Rhl(Rhl I and Rhl R) system. iii) Azithromycin and Tobramycin etc. are known inhibitors of QS. iv) Anti-QS activity have also been shown by *Conocarpus erectus, Callistemon viminalis* and *Bucida bucerus* etc.
3.	Type III secretory system(T3SS)	i) T3SS is an injectisome i.e. belongs to the Psc secretory system spanning from inner membrane to extracellular space. ii) Enhances pathogenesis by injecting effector toxins (Exo S, Exo T, Exo U and Exo Y) into the host cell. iii) β- Lactams exhibited T3SS inhibitory activity.
4.	Exoenzyme S	i) Exo S is an effector toxin protein showing GTPase activity thereby accelerating host cell mortality by inactivating Rho, Rac1 and Cdc42 proteins responsible for regulating the shape and intracellular movement of the cell. ii) It also affect the intracellular signal ransduction pathway in infected host cell by ADP- ribosylation of Ras and Rab proteins iii) Aminoglycosides, fluoroquinolones and cephalosporin have been reported as inhibitor of such protein.
5.	Exotoxin A	i) It is an extracellular enzyme made up of single polypeptide chain which accelerates pathogenesis by affecting translation in the host cell by ADP ribosylation of elongation factor-2 ii) Amikacin is a well known inhibitor of such protein

Table 2: Preferred Herbals and their related importance

S. no	Herbal plant	Ethnopharmacological importance	Relevance of herb in traditional medicine	Availability	Evidences of their use from Vedic literature	Prior investigation
1	***Barberine vulgaris***	Bark extract is used for liver malfunctions, gallbladder disease, jaundice, splenopathy, indigestion, respiratory infection, piles, renal disease, gout, rheumatism, malaria and leishmaniasis	Relieves constipation and stimulate appetite	Europe, northern Africa, parts of America and Central Asia	Reported in Ayurveda	Reported to be anticarcinogenic, antidiarrhoeal, antipyretic, antimicrobial, anti-inflammatory etc.
2	***Vaccinium macrocarpon***	Riped fruit are used to cure urinary tract infection	Juice is used to kidney stone and skin infection	Native to north America and Asia	Reported in Ayurveda and Unani	Reported to be antioxidant,antimicrobial and used to treat cardiovascular diseases
3	***Zingiber officinale***	The plant have shown significant medicinal role as anti-inflammatory and chemoprotective agent also used to cure respiratory and urinary ailments	Reported as a drug against arthritis, vomiting, migraine and as natural pain reliever	Native to Asia and cultivated in various countries including India	Reported in Ayurveda	It has been reported to be useful against urinary tract infection, respiratory tract infection, digestive disorder and pharmacologically in drug form it is used as appetizer
4	***Matricaria recuitita***	Reported to have antioxidant, anticancerous properties	Its oil is used as a antispasmodic, antimicrobial and anxiyolytic agent	Found in Europe and North America and Australia	Reported in Ayurveda	Accounted to have potential antiviral properties against herpes simplex virus type-2
5	***Trachysper mum ammi***	Fruit is popularly used to treat bronchial problem, asthma, lack of apetite, abdominal tumors, piles etc.	Traditionally used to cure diarrhoea, atonic dyspepsia, amenorrhoea etc.	Native of India, Pakistan, Iran and Egypt	Used for antitussive, diuretic, homeostatic, laxative agent. Also to cure sore throat, cough and during diarrhoea	Medicinally it has been proven to possess pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic, hypolipidemic, antihypertensive has broncho-dilating actions
6	***Oxycoccos palustris***	Fruit is used to cure skin infection, urinary tract problems and kidney stone	Riped fruit are used as urinary tract infection, bladder infection.	Native to north America and Asia	Reported in Ayurveda and Unani	Reported to be antioxidant, antimicrobial, and to treat cardiovascular diseases
7	***Aloe barbadensis***	It has anti inflammatory, antioxidant, antimicrobial, anticancer, antidiabetic, immuneboosting and hypoglycemic properties	Used against cancer by activating macrophage and also inhibits metastasis	The genus is native to Africa	Medicinal use is recorded in Greek, Roman and Ayurveda	Scientific studies exist that support the antibacterial and antifungal effect of aloe vera. Also reported to be useful in burns, frostbite and skin abrasion injury

8	***Terminalia chebula***	Used to treat, urinary tract infection, artherosclerosis, anaphylactic shock, to improve gastrointestinal activity and have antispasmodic activity, antidiabetic activity and also useful in wound healing.	Used as mild laxative and as astringent against wounds and abscesses. In dental care, against ulcer of gum and the plant is use as antidote and against snake bite and in respiratory tract infection	Native of India and Nepal, China, Malaysia and Vietnam	Reported in Ayurveda and Chinese medicine	Pharmacologically reported to cure bronchial asthma, cough, diarrhoea etc.
9	***Azadirachta indica***	Complete plant is used to cure different ailments such as urinary tract and respiratory tract infecton, jaundice, stomach ulcers and variety of infectious diseases including leprosy to chicken pox	Neem seed oil and essential oil from bark and leaves have been reported to show activity against different pathogenic bacteria like *Pseudomonas aeruginosa* and *Streptococcus etc.*	Native to India, Pakistan and Bangladesh	Reported in Ayurveda and Unani medicine system	Many arthropod ectoparasites are susceptible to growth regulatory, insecticidal and insect repellant activity of neem extract
10	***Boerhaavia diffusa Linn***	Leaves used to cure asthma, urinary disorders, leucorrhea, rheumatism and encephalitis, stem and roots are effective drugs to cure acute promyelocytic and leukemia	It is used to treat different disease such as cancer, urinary tract and respiratory tract infections	Native of Australia and Asia	Reported in Ayurveda and Unani	It shows anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, anticancer, antiestrogenic, immunomodulatory and antiamoebic activity
11	***Bacopa monnieri***	Whole plant is used to cure the anxiety, epileptic disorder and hypothyroidism	It is used to treat respiratory tract and urinary tract infection, irritable bowel syndrome	Native to Southern India and Australia	Reported in Vedic and Ayurveda	Used as antimicrobial, antioxidant, antigastric, anticancer and memory enhancer
12	***Asparagus officinalis***	Rhizome and stem are used to treat reproductive diseases, urinary tract diseases, respiratory tract diseases	It is used to treat hepatitis, rheumatism and gout	Native to Asia and Europe	Reported in Vedic, Ayurveda and Chinese medicine	Used for anti-inflammatory, Production of red blood cell, urinary tract and respiratory tract infection
13	***Ocimum tenuiflorum***	Different parts effective against bronchitis, bronchial asthma, malaria, diarrhea, dysentery, painful eye disease, chronic fever etc.	The plant possess antifungal, anticancerous, antidiabetic, anti-inflammatory, antifungal, hepatoprotective, antispasmodic and analgesic action	Native to India	Reported in Ayurveda	Useful in diarrhoea, dysentery, hemorrhage, hemmarhoids, hepatitis and skin diseases
14	***Coriandrum sativum***	The plant has a very effective antioxidant profile	Possess hepatoprotective activity	Native of Southern Europe	Reported in Ayurveda	Plant have therapeutic potential due to the presence of natural antioxidants
15	***Abutilon indicum***	Various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic and analgesic	The roots of the plant are considered as demulsant, diuretic, in chest infection and urethritis	Native to tropic and subtropical regions	The plant is very much used in Siddha medicines	Reported to treat leprosy, ulcers, headaches, gonorrhea and bladder infection
16	***Andrographis paniculata***	Use to treat upper respiratory infection, ulcerative colitis and rheumatic symptoms	The herb had a significant drying effect on the nasal secretions of cold suff-erers	Native of Asia	The plant used in traditional Siddha and Ayurveda	Have potential to be used as a mosquito repellant

17	***Plantago ovate forssk***	Mainly use as a dietary fiber	Significant role as a laxative	Native to Europe	Reported in Ayurveda	As a dietary supplement in the management of hypercholesterolaemia and to reduce the risk of coronary heart disease
18	***Bauhinia variegate***	Used as potent agent for antidiabetic, anti-inflammatory, antitumor, liver protective, antibacterial, haemagglutinating, haematinic and antiulcer infection	Used in treating stomach related problems, ulcers, cyst and tumors	Native to South Asia	Reported in Ayurveda	Reported to be useful as antidiabetic agent
19	***Flacourtia indica***	The bark is believed to be effective for arthritis	The leaves and roots are used in herbal medicine for treatment of snakebite	Native to Africa and Asia	Reported in Ayurveda	Most parts of the plant are used for cough, pneumonia and bacterial throat infection
20	***Juniperus communis***	Juniper is used for digestion problems including upset stomach, heartburn, bloating, as well as gastrointestinal (GI) infections and intestinal worms	It is also used for urinary tract infections (UTIs), kidney and bladder stone s	Native to America	Reported in Ayurveda	Reported to treat bronchitis
21	***Euphorbia ligularia***	Used to cure asthma, earache, rheumatism and toothache	Leaves and fruit are use as astringent, anthelmintic laxative agent	Native to Asia	Reported in Ayurveda and Siddha	Reported to be used in asthma, bronchitis, jaundice, cutaneous infection etc.
22	***Hydrangea macrophylla***	Roots and rhizomes are used to treat urinary tract problems and hay fever	Useful for wound healing and prostate disorders	Native to South America and Asia	Reported in Chinese medicine	It is used as antimalarial, antitussive and diuretic
23	***Foeniculum vulgare***	Seeds are useful for the treatment of gastrointestinal disorders, and fennel extracts are known to have estrogenic properties	It is a successful appetite enhancer and helpful in relievement of abdominal cramps gas	Native to Europe, India and Persia	Reported In Chinese Medicine and Unani	It is used to treat asthma, bronchitis and cough
24	***Armoracia rusticana***	Use of its root is well known for antibacterial, antirheumatic and antiseptic activities	The plant is antibiotic against gram-negative and gram-positive bacteria and also pathogenic fungi	Native to southeastern Europe and western Asia	Reported in Chinese medicine and Unani	It is experimentally proved to have antitumor and antioxidant properties
25	***Taraxacum officinalis***	Use to treat kidney disease, swelling, skin problem, heartburn and upset stomach	Used to treat stomach problems, appendicitis and breast problems	Native to Europe, Asia and north America	Reported in Chinese medicine	Used as remedy for fever, eye problem, diarrhoea
26	***Mentha piperita***	Therapeutic potential of aromatic herbs in the treatment of respiratory ailments	It has been proven helpful in symptomatic relief of the common cold	Native to Europe	Reported in Ayurveda	Used to treat coughs, bronchitis, and inflammation of the oral mucosa and throat
27	***Agathosma betulina***	Leaves are useful to treat cold, Urinary tract infection, stomach ailments etc.	Used to treat respiratory disorder, sinus problem, kidney infection, premenstrual syndrome and prostate disorder	Native to south Africa	Reported in Ayurveda and traditional Chinese medicine	It have been used as diuretic agent
28	***Althea officinalis***	Complete plant especially the roots are useful to treat respiratory, alimentary and urinary organs	Used to treat pneumonia, kidney stone and acute dysentry	Native of Europe	Reported in Ayurveda and Chinese medicine	Reported to cure asthma, bronchitis, cold, cogh, stomachache and skin irritation

29	***Achillea millefolium***	The whole herb can be used as a medicinal agent to treat cold, influenza and stomachache	Used as anti inflammatory, antiseptic, and astringent	Native of Europe, Asia and north America	Reported in Ayurveda	This genus include treatment of wounds, bleedings, headache, inflammation, pains, flatulence and dyspepsia
30	***Arctium lappa***	Roots are mainly used to treat respiratory disorder, urinary ailments, joint pains etc.	Use to treat acne, ulcers and tonsilitis	Native of Europe, Asia and north America	Reported in Ayurveda and traditional Chinese medicine	Reported to be used as blood purifier and antioxidant agent
31	***Nasturium officinale***	Useful to treat respiratory disorder like cold, cough and bronchitis	Used to treat hypertension, hyperglycemia and renal problem	Native of Europe, Asia and north America	Reported in Ayurveda and Chinese medicine	Reported to be useful against Multi-drug resistant tuberculosis
32	***Papaver somniferum***	Fruit is used to treat pain	Used as analgesic	Native of Asia	Reported in Ayurveda and traditional medicine	Useful to treat insomnia
33	***Sambucus nigra***	Use to cure tonsillitis, cold, cough, Urinary disorder and arthritis	Use as a blood purifier	Native to Europe, northwest Africa, southwest Asia and America	Reported in Ayurveda	Dried root is an excellent herbal remedy for dropsy
34	***Cinnamomum cassia***	Dried bark is used for cough ,cold and diarrhoea	Used to treat infection caused by *Staphylococcus aureus* and *Pseudomonas* aeruginosa	Native to China and Srilanka	Reported in Chinese medicine	Itis antirespiratory antimicrobial agent
35	***Glycyrrhiza glabra***	Plant is used for its ethnopharmacological values to cure various ailments like cough, hepatitis, acute respiratory syndrome and cancer	Useful for the digestive and respiratory disorder	Native to Asia	Reported in Unani and Ayurveda	It is used as a antidiabetic, antimicrobial, antioxidative, anticancerous and hepatoprotective agent
36	***Eucalyptus globus***	Dried leaves and oil is used for cough, cold and upper respiratory system	Useful for the treatment of dental care, wound healing, analgesic properties	Native to Australia, India	Reported in Ayurveda	It is used as Antimicrobial ,anti-inflammatory and stimulating immune system
37	***Allium sativum***	Small cloves have been used for antibacterial, antimycotic and lipid reducing effects	Used in bronchitis, constipation, joint pain and fever	Central to southern Asia	Recorded in Ayurveda, from ancient Chinese to Egyptian culture	Allicin, the active compound is antimicrobial, lipid reducing, anti oxidative and fibrinolytic
38	***Hibiscus rosasinensis***	Used in folk medicine for the treatment of pain and various inflammatory conditions such as the inflammation of oral mucosa, blenorrhoea, and asthmatic bronchitis	Diuretic, laxative, lowering blood pressure and curing cough. Also used for hair care (against loss and greying)	Native of east Asia	Reported in Ayurveda	The plant have significant anti-inflammatory activity

Table 3: Binary Score Matrix of Herbals based on Bioactivity Parameter

S.No	Plants	Bioactivity Parameter					Total Binary score
		Biofilm formation inhibition	Quorum sensing inhibition	Type III secretion system inhibition	Exoenzyme S inhibition	Exotoxin A	
1	***Barberine vulgaris***	1	1	1	0	0	3
2	***Vaccinium macrocarpon***	1	0	0	0	0	1
3	***Zingiber officinale***	1	1	0	1	0	3
4	***Matricaria recuitita***	1	0	1	0	1	3
5	***Trachyspermum ammi***	0	0	0	0	0	0
6	***Oxycoccos palustris***	1	0	0	0	0	1
7	***Aloe barbadensis***	1	1	0	1	1	4
8	***Foeniculum vulgare***	1	1	1	0	0	3
9	***Ocimum tenuiflorum***	0	0	0	0	0	0
10	***Terminalia chebula***	1	1	1	0	1	4
11	***Coriandrum sativum***	0	0	0	0	0	0
12	***Abutilon indicum***	0	0	0	0	0	0
13	***Boerhaavia diffusa Linn***	1	0	0	0	1	2
14	***Andrographis paniculata***	0	0	0	0	0	0
15	***Plantago ovate forssk***	0	0	0	0	0	0
16	***Bacopa monnieri***	1	0	0	1	1	3
17	***Bauhinia variegate***	0	0	0	0	0	0
18	***Flacourtia indica***	0	0	0	0	0	0
19	***Juniperus communis***	0	0	0	0	0	0
20	***Euphorbia ligularia***	0	0	0	0	0	0
21	***Asparagus officinalis***	0	0	0	1	0	1
22	***Armoracia rusticana***	0	0	0	0	0	0
23	***Taraxacum officinalis***	0	0	0	0	0	0

24	***Mentha piperita***	1	1	1	1	0	4
25	***Agathosma betulina***	0	0	0	0	0	0
26	***Hydrangea macrophylla***	0	0	0	1	1	2
27	***Althea officinalis***	0	0	0	0	0	0
28	***Achillea millefolium***	0	0	0	0	0	0
29	***Arctium lappa***	0	0	0	0	0	0
30	***Azadirachta indica***	0	1	1	1	1	4
31	***Cinnamomum cassia***	1	0	0	1	0	2
32	***Nasturium officinale***	0	0	0	0	0	0
33	***Glycyrrhiza glabra***	1	1	1	1	1	5
34	***Papaver somniferum***	0	0	0	0	0	0
35	***Eucalyptus globus***	0	1	0	1	0	2
36	***Sambucus nigra***	0	0	0	0	0	0
37	***Allium sativums***	0	1	0	1	0	2
38	***Hibiscus rosasinensis***	0	0	0	0	0	0

Table 4: Fuzzy Score Analysis of Herbal Leads

S. No	Herbal Leads	Weightage Score [S]	μs*	Optimization Score#
1.	*Glyccyrhiza glabra*	15.42	1	+++++
2.	*Terminalia chebula*	13.92	0.773	++++
3.	*Aloe barbadensis*	13.4	0.694	+++
4.	*Mentha piperita*	13.12	0.652	+++
5.	*Foeniculum vulgare*	11.9	0.468	++
6.	*Zingiber officinale*	11.73	0.440	++
7.	*Berberis vulgaris*	11.62	0.425	++
8.	*Azadiracta indica*	10.42	0.244	+

* μS = [(S) – min(S)] / max(S) – min(S)], where μS is the Fuzzy value and [S] is the Weightage matrix score; Max(S) = 15.42; Min (S) = 8.82 (*Bacopa monnieri* and *Matricaria recuitita not shown in Table as* μS= 0 and μS= 0.078 respectively*)* ; Herbal Leads with Binary Matrix Score ≥3. # Optimized Score Range- 0.10 – 0.30 = +; 0.30 - 0.50 = ++; 0.50 - 0.70 = +++; 0.70-0.90 = ++++; 0.9- 1.0 = +++++