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Herbal Informatics Approach for targeting Nosocomial Infections caused by *Klebsiella pneumoniae*

Pallavi Thakur[1], Raman Chawla[1*], Sana [1], Rajeev Goel[1], Alka Narula[2], Rajesh Arora[3], Rakesh K. Sharma[1]
[1]Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Delhi - 110054, India
[2]Department of Biotechnology, Jamia Hamdard, Delhi - 110062, India
[3]Office of DG (LS), Defence Research and Development Organisation, DRDO Bhawan, New Delhi - 110011, India
*Corresponding author: Raman Chawla

Abstract

The classical herbal bioprospection is identification of herbal medicinal plants based on its ethnopharmacological importance, as testified in ancient literature or otherwise in clinical literature of various countries. This process is time consuming, tedious, generally observation or experience based, and might lack scientifically evident and validated proofs. Evolution of new techniques of deploying dynamic search protocols, priority indexing, systemic categorization and cross-verification could be referred to as an *in silico* bioprospection tool. The parallel research efforts globally on both herbals and antimicrobials provides enormous web based data that requires to be filtered systematically towards a logical conclusion for further *in vitro* and *in vivo* validation. The present study aims to simulate the above referred models utilizing *in silico* herbal bioprospection modeling, literature based parameter selection, priority indexing using random search model, scoring and decision matrix based analysis followed by optimization and validation.

Keywords : Herbal Bioprospection, Nosocomial, Klebsiella pneumoniae, Multi-Drug Resistance, Ethnopharmacology

Introduction

Plant products play an important role in the health care systems of developing countries like India where it serves as a source of both traditional and modern medicines for fighting against infectious diseases. Over the years, World Health Organization (WHO) advocated traditional medicines as safe remedies for ailments of both microbial and non-microbial origins[1]. There are about 3, 50,000 species of plants growing on the earth and it is estimated that at least 5000 different chemical compounds are present in a single species of plant. It is apparent that the secondary metabolites of plant origin constitute a tremendous resource for exploring useful drugs. Secondary metabolites (SM), which plants employ to defend themselves against bacteria, fungi or viruses, can be used in almost the same way in medicine to treat microbial or viral infections[2].

Some of the most powerful and potent drugs used nowadays were derived from plants, suggesting the importance of herbal plants as a therapeutic modality. The modern microbiological techniques demonstrate that higher plants frequently exhibit significant potency against human bacterial and fungal pathogens[3]. Use of herbal extracts having antimicrobial potential can be of great significance for finding lead new compounds to treat human pathogenic diseases, like pneumonia, tuberculosis, diarrhoea, MRSA (Methicillin Resistant *Staphylococcus aureus*) & NDM-1 (New Delhi Metallo-beta-lactamase-1) *Escherichia coli* infections. Contrary to synthetic drugs, antimicrobials of plant origin are not associated with serious side effects and have an enormous therapeutic potential to treat many infectious diseases with better acceptability and ease of availability in particular geographical region.

Nosocomial infections pose a serious health problem resulting in an enormous burden of morbidity and mortality rates, and high health care costs. Studies have indicated that nosocomial infections occurred in 5-10% of all hospitalized patients in Europe and North America and in more than 40% in parts of Asia, Latin America, and sub-Saharan Africa[4]. At any time, over 1.4 million people worldwide suffer from infectious complications acquired in hospital[5]. A study in India on 629 patients of Intensive Care Unit, revealed the rate of nosocomial infections as 21%, and the most common infection was reported pneumonia (with a rate of 29.5%) with *Klebsiella pneumoniae* detected as the most common pathogenic microbial agents[6]. Infections with Gram negative bacteria like *Klebsiella pneumoniae* are of imminent concern as they are more difficult to treat and visual outcome is poor. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of such diseases. More recently, the emergence of carbapenemase producing *K. pneumoniae* (KPC) has severely challenged antimicrobial therapy, since it confers a high level of resistance to all β-lactams and distinct levels of resistance to carbapenems[7].

It is therefore essential to develop a systematic and standardized approach to bioprospect, identify, test and validate the herbal candidates as potential therapeutic leads, using both *in silico* and *in vitro* approaches in conjunction. The classical herbal bioprospection is identification of herbal medicinal plants based on its ethnopharmacological importance, as testified in ancient literature or otherwise in clinical literature of various countries. This process is time consuming, tedious, generally observation or experience based, and might lack scientifically evident and validated proofs[8]. Evolution of new techniques of deploying dynamic search protocols, priority indexing, systemic categorization and cross-verification could be referred to as an *in silico* bioprospection tool [9,10,11]. The parallel research efforts globally on both herbals and antimicrobials provides enormous web based data that requires to be filtered systematically towards a logical conclusion for further *in vitro* and *in vivo* validation.

The present study aims to simulate the above referred models utilizing *in silico* herbal bioprospection modeling, literature based parameter selection, priority indexing using random search model, scoring and decision matrix based analysis followed by optimization and validation. Such tool can be used to validate findings of the classical bioprospection. This study has provided an insight into a systematic collection and analysis of literary data to obtain a logical output for ascertaining a desired biological activity. The validation step involves the analysis of the docking potential of predominant phytoconstituents against extended spectrum beta lactamase.

Methodology

Selection of microorganism

Microorganisms to be targeted using alternative system requires to follow some of the important characteristics i.e., a) lethal, sub-lethal, incapacitating or potentially dangerous Biothreat agent; b) either no treatment regime/vaccine available or limited availability; c) evolving virulent forms from past; d) possibly could be used as bioweapon which are lethal and/or panic creating agent.

Selection of bioactivity parameters using classical approach

The holistic mitigation requires multi-targeted approach. Based on the understanding of the mechanistic aspects of antibiotic resistant patterns of micro-organisms, as in present study, nosocomial infections harbored *Klebsiella pneumoniae,* the various comparable targets attributing towards bactericidal activity of *Klebsiella pneumoniae* has been selected on the basis of extensive literature surge (Classical Bioprospection Approach). There are certain parameters which need to be assessed to analyze the bioactivity associated with a given herbal plant, with respect to its potential of treating dreadful infections allied with virulent multidrug resistant bacterial strains, like *Klebsiella pneumoniae*. The six testing parameters were selected for study based on mechanistic aspects of antibiotic resistance of *Klebsiella pneumoniae* harboring strains, including extended spectrum beta lactamase inhibition, MDR efflux pump Inhibition, adhesion inhibition, capsular polysaccharide inhibition, siderophore inhibition and symptomatic relief provision[12, 13].

Evaluation of relevance factor using keywords hits scoring matrix approach

The analysis was conducted using PubMed as selected search engine. The random search model using combination keyword as Bioactivity Parameter + Antibacterial activity while advanced search model using the same combination keywords but in quotes, yielded 'N' hits. The first n=20 hits provided by the search engine, working on the principle of priority indexing, were based on the number of times a website is read/clicked. The first 20 hits are subjected to observational interpretation for assessing relevance using human interface. This sample set based analysis was used to evaluate the net weightage linked to each bioactivity parameter, using the following formula:

{% (Relevance) avg = (No. of relevant hits based on observational analysis) X 100 / (n=20)}

Selection of herbal plants using classical bioprospection approach

The classical bioprospection approach accounts for investigation of the following variables based on literature review to devise a logical conclusion, resultant in selection of plants. It includes a) Ethnopharmacological importance of plant; b) Relevance of Herb in traditional medicine; c) Availability factor or cultural acceptability in localized regions; d) Any vedic literature supporting its use; e) Investigations/ prior experience on potential of the herb; f) Indirect indications, if any etc. The final conclusion to select a plant for *in silico* bioprospection is based on learning of the subject area conjugating with prior experiences/ investigations[14-26]. 44 herbals selected on the basis of ethno-pharmacology were used as test set.

Binary coefficients matrix to evaluate the presence/absence of a parameter in selected plants

This methodology works on the principle of 0-1 binary code of absence/presence of a particular parameter in selected plants from previous step. The range of outcome of matrix lies between 1 to 6 for any plant. The cut off value selected for this matrix based analysis is closest value to the median of 1-6 range. Based on this, all the plants having more than 03 parameters, reported in PubMed search engine (n= first 20 hits) against 'Bioactivity Parameter + Selected Plant' random search model, were selected. It relates to the fact that only these plants which can support holistic approach should be screened for the next level analysis, in line with the rationale of present study.

Weightage matrix based analysis

This step includes evaluation of overall weightage of plants (Scores ≥3 in previous step) by multiplying their binary score with weightage obtained in above step. This is a primary step to screen the plants utilizable to subsequent analysis and removes fake positive results attributed towards investigator's biasness due to 'experience factor'. This step enhances the 'uncertainty factor' required for statistically valuable outcome. This step identifies potential plant leads based on *in silico* bioprospection approach subjected to fuzzy set membership analysis and optimization to validate the findings.

Fuzzy set membership analysis for decision matrix

In this approach, the given mathematical relationship was used to calculate the relevance of the variety/product;

$$\mu S = S\text{-}min(S)/[max(S)\text{-}min(S)]$$

where: μS represents the desirability values of members of the fuzzy set S. Min(S) and max(S) are minimum and maximum values, respectively, in the fuzzy set S.

Optimization of 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.

Results

Keywords hits scoring matrix

On the basis of the keyword hits scoring analysis, weightage was given to various parameters identified for screening of herbal plants with respect to antimicrobial activity, as exemplified in Table 1. Weightage was decided according to the percentage relevance obtained for each parameter, as elucidated in Figure 1. Highest relative percentage relevance was obtained for capsular polysaccharide inhibition (CPI) (i.e. 6), followed by other parameters like Extended Spectrum Beta Lactamase Inhibitor (ESBLI) (5.9), MDR Efflux Pump inhibition (EPI) (5.6), Adhesion Inhibition (AI) (4), Siderophore Inhibition (SI) (3.6), and Symptomatic Relief (SR) (3.2).

Binary (presence-absence) coefficients matrix

Out of 44 identified herbals, 18 herbals exhibited a binary score of either 3 or more than 3, significantly ($p<0.05$) higher than that of the counterparts, e.g. *Punica granatum*, Glycyrrhiza glabra, *Thymus vulgaris, Rosemarinus officnalis, Ocimum sanctum, Terminalia chebula, Mentha piperita, Cucuma Domestica, Brassica nigra, Allivum sativum, Syzgium aromaticum, Psidium guajava, Sesame spp, Bridella micrantha and Pongamea pinnata, Artemisinin annua, Camellia sinesis*. Ethnopharmacological relevance behind the selection of various herbal plants was also exemplified in Table 2.

Simple additive weighing matrix

Out of 18 plants selected on the basis of binary coefficient matrix (Binary Matrix score ≥ 3), it was revealed that 6 herbal plants showed immense potential of acting as a therapeutic agent against drug resistant microorganisms, as their combined weightage scores were significantly ($p<0.05$) higher than the median value score i.e. 18.5, e.g. *Rosmarinus officinalis*, *Punica granatum* etc. as shown in Figure 2.

Decision matrix based Optimazation

On the basis of decision matrix analysis, 6 plants were found to show high percentage relevance to be chosen as potent therapeutic herbal plants against drug resistant bacteria, as shown in Table 3. Amongst these, *Rosmarinus offcinalis* held the topmost position with μS score being 1, relative to the lowest μS score exhibited by *Glycyrrhiza glabra* (0.72).Optimized scores were also obtained for the selected plants based on fuzzy set membership analysis.

Discussion

The present study revealed 06 herbals (**H-01 to H-06** as explicated in table-3) as potent candidates to be explored for their holistic abilities against MDR *K. pneumoniae* (Table-3). The analysis of presence of pre-dominant phyto constituents in these herbals revealed Rosmarinic acid & Curcumin (phenolics in **H-01** and **H-02**); 6” – O- (E) – feruloylhomoplantaginin, Quercetin & Demethoxycurcumin (flavonoids in **H-01, H-02** and **H-04**); Carnosic acid (Diterpene in **H-01**); Gallic acid &

Ellagic acid (Tannins in **H-03**); Licochalcone A (Isoflavones in **H-05**); Glycyrrhizin & Glabridin (Glycosides in **H-05**); Carvacrol & Thymol (Essential oils in **H-06)** and; Oleoresins (**H-02)**[27-30].

The primary mechanism of averting multidrug resistance conferred by *K. pneumoniae*, *Bacillus subtilis* and *Streptococcus mutans* etc. include: (a) Disruption of membrane – (i) proton permeability; (ii) lipid bi layer partitioning; (iii) precipitation of membrane proteins and; (iv) active release of lipo-polysaccharides (b) Inhibition of co-aggregation of micro-organisms due to disruption of quorum sensing and; (c) modifications in essential growth parameters of bacteria – (i) disruption of core metabolic pathway of folic acid metabolism (shikimate dehydrogenase) thereby altering bacterial cell division; (ii) Unavailability of Fe, a trace element of host body acts as essential nutrient for bacterial growth [28,30, 31, 32].

Carnosic acid (**H-01**) & Rosmarinic acid (**H-01**) were reported to avert multi drug resistance by enhancing proton permeability thereby disrupting membrane potential & enhanced drug influx while Curcumin (**H-02**) & Demethoxycurcumin(**H-02**) interacts with membrane proteins thereby enhancing lipid bi layer partitioning & fluidity. On the other hand, Gallic acid & Ellagic Acid(**H-03**) precipitation of protein due to their phenoxyl hydroxyl radicals while Essential oils (**H-06**)**-** thymol and carvacrol, essential oil induces the active release of lipo-polysaccharides by hydrophobic interactions with gram negative bacterial cell wall [33].

Curcumin (**H-02**) & Demethoxycurcumin (**H-02**); Gallic acid & Ellagic Acid (**H-03**)along with lectins (**H-04**), were reported to be quorum sensing inhibitors by altering cellular morphology through inactivation of microbial adhesions, enzyme complexes and transport proteins[31]. Such process led to inhibition of ability of bacterial cell to interact with host cell as well as intra-cellular communication responsible for colonization.

Curcumin (**H-02**) was also able to disrupt folic acid metabolism while lectins (**H-04**) were reported to chelate Fe, thereby limiting its availability for bacterial cell growth. *Glycyrrhiza glabra* contains Glycyrrhizin and glabridin (**H-05**) which may inhibit the generation of reactive oxygen species (ROS) by neutrophils at the site of inflammation. Also, licorice isoflavones, hispaglabridin A & B (**H-05)**, inhibit (Fe.sup.3)-induced mitochondrial lipid per-oxidation. Further the mechanism of antimicrobial activity is also attributed to licochalcone A which inhibits NADH Cytochrome c reductase of bacterial electron transport chain.

The presence of multiple constituents working synergistically and / or antagonistically in herbals led to desired antibiotic resistance modification. Such process can be associated with antagonistic behaviour of essential oils towards similar target thereby reducing overall efficacy. *Curcuma domestica* (µS = 0.9 & optimized score~ 5; Table-3) have been reported to contain pre-dominant phyto-constituents Curcumin; Demethoxycurcumin. The analysis revealed that these phyto-constituents are able to illicit numerous physiological responses altering antibiotic resistance. This model hence provides a process to identify novel chemical moieties of natural origin as a strategic preventive measure for any forthcoming biological threat. The selected herbs / constituents are required to be evaluated *at in vitro* and *in vivo* level to identify ‘ drug –like moieties’ in the coming future.

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Tables and Figures

Table 1. Rationale for Selection of the Bioactivity parameters for Bioprospection Study

S.No	Bioactivity Parameter (BAP)	Rationale for selection
1	Capsular Polysaccharide inhibition (CPI)	a. *Klebsiella pneumoniae* possesses K2 type of virulent capsular polysaccharide made up of mannose sugar which prevents its recognition by macrophage, thereby preventing phagocytosis. b. *Rosemarinus officinalis, Glycyrrhiza glabra* and *Thymus vulgaris* are known to enhance cell wall permeability.
2	Extended Spectrum beta lactamase inhibitor (ESBLI)	*a. Klebsiella pneumoniae* possesses Extended-spectrum beta-lactamases (ESBLs) with an ability to hydrolyze third-generation cephalosporins and aztreonam yet are inhibited by clavulanic acid. b. *Dortenia picta, Psidium guajava* etc have been reported to exhibit β-lactamase inhibition activity and can be used as antibiotic potentiation against resistant bacterial strains.
3	Efflux Pump inhibitor (EPI)	a. Increased efflux decreases the intracellular concentration of the antimicrobials, thereby allowing bacterial survival for a greater length of time. b. Use of EPIs like *Thymus vulgaris* against AcrAB efflux pump of *Klebsiella pneumoniae* could facilitate the potentiation of antibiotics like ciprofloxacin and might even suppress the emergence of such MDR strains.
4	Adhesion inhibition (AI)	a. Lipopolysaccharide based Biofilm formation is one of the virulent factors of *Klebsiella pneumoniae* which promotes host colonization by increasing uroepithelial adhesion using Type 2 fimbriae. b. Plants like *Terminalia chebula* and *Mentha piperita* inhibits epithelial adhesion of *Klebsiella pneumoniae* thereby preventing colonization.
5	Sidereophore inhibition (SI)	a. Iron acquisition systems based on siderophores allows systemic growth of *Klebsiella pneumoniae* by secreting iron-chelating compounds with extremely high Fe^{3+} affinity. b. *Curcuma domestica* and *Ocimum sanctum* are known to inhibit these iron acquisition systems by showing competitive Fe^{3+} chelation.
6	Symptomatic relief (SR)	a. Klebsiella associated Urinary Tract infections, Gastroenteritis, Nosocomial Pneumonia, Bacteremia, Wound infection and transient fever are treated using these phytochemical based modalities like *Eucalyptus globules* (pain relieving); *Aloe barbadensis* (Anti-inflammatory); *Ocimum sanctum* (Anti-pyretic); *Plumbago zeylanica* (controlling Urinary Tract Infections) and *Allium sativum* (controlling bacteremia) etc.

Table 2: Selected Herbal plants showing probable utility against *Klebsiella pneumoniae* infections

S.No	Herbal Plant	Common name	Predominant Phyto constituents	Parts utilized	Availability	Relevance of Herb in Traditional Medicine	Relevance in Vedic Literature	Current Indications
1	*Allivum sativum*	*Garlic, Stinking Rose*	Allicin, ajoenes allyl disulfide, allypropyl disulfide	Bulbs clove	Extensively grown in Batangas, Nueva Ecija, Southern Europe, cultivated in all parts of world	Arthritis, rheumatism, toothaches, digestive problems and gastrointestinal spasms	Mentioned in Ayurveda, Chinese traditional medicines	Antibacterial, antihelminthic, antimycotic, antiviral, antispasmodic, diaphoretic, expectorant, fibrinolytic, hypotensive, promoting leucocytosis, lipid lowering and platelet aggregation inhibition.
2	*Artemesinin annua*	Sweet worm wood, sweet annie	Artemisinin, arteether, artemether, artemotil, artenimol, artesunate and dihydroartemisin	Dried aerial parts	Grown in China, Japan, Germany, Korea	Aqueous preparations of the dried herb were applied against fever, malaria, skin diseases, jaundice and haemorrhoids	Traditional Chinese medicine	Antiangiogenesis effects, Antimalarial effects, Antioxidant, Anticancer, Antimicrobial and Antiangiogenesis effects
3	*Brassica nigra*	Black mustard	Phytoalexins (sinalexin, sinalbins A and B), sterols and steryl esters (primarily sitosterol and campesterol), and flavonoids (eg, apigenin, chalcone).	Dried seeds	Native to the southern Mediterranean re gion of Europe and possibly South Asia	Food flavoring, for forage, as an emetic, and diuretic, as well as a topical treatment for inflammatory conditions such as arthritis and rheumatism.	Ayurveda	Antibacterial, Hypergylcemic and cardiovascular effects
4	*Bridella micrantha*	Coast gold leaf	Benzene, 1.3-bis 2-Pinen-4-one, 1,8 Cineole, camphor, α-Pinene, borneol	Stem bark	Native to primarily tropical, northeast, western, west-central, and Southern Africa	Used locally in folk medicine, variously as an anti-abortifacient, an antidote, a laxative or purgative; and to treat diverse conditions of the central nervous system (headache), eye (infections , conjunctivitis), gastrointestinal system (abdominal pain, constipation, gastritis), respir atory system (common cold	Mentioned in Ayurveda	Antiamebic, antianemic, antibacterial, anticonvulsant, antidiabetic, antidiarrheal, antihelminthic, anti-inflammatory, antimalarial, antinociceptive, antiviral, and hypoglycemic effects
5	*Camella sinesis*	Tea	Caffeine, Theobromine, Theophylline, Purine derivatives like xanthine, methylxanthine, and adenine, tanning agents (tannin, polyphenols, gallic acid, and catechin derivatives)	Shrub	Originally from the triangle of countries of South China, Assam (northeastern India) and Cambodia. - Planted in almost all tropical and subtropical regions of the world.	Promote blood circulation, promote excretion of alcohol and other harmful substances, invigorate the skin, promote digestion, combat tiredness and depression, among many others. Strong infusions were used as external applications for skin ailments, eruptions, abrasions and athlete's foot.	Recorded as early in the 6th century as a Chinese herbal medicine, recommended particularly for people who slept too long. It was used to promote	Anti oxidant, anti diabetic, hypolipidemic, antibacterial, hepatoprotective
6	*Curcuma domestica*	Long turmeric	Curcumin (diferuloylmethane) and various volatile oils, including tumerone, atlantone, and zingiberone.	Rhizome, leaves	Widely distributed in the Philippines Native of India Now pantropic	Decoction of rhizome, as tea, used for fevers, dysentery, abdominal pain, flatulence, abdominal spasm, arthritis. In the Philippines, rhizomes with coconut oil used as stomachic and vulnerary.	Ayurveda, Malays in China	Antioxidant, antiinflammatory, cholesterol-lowering, antibacterial, antifungal, antiviral, immunomodulatory,

						Internally, juice of fresh rhizome used as anthelmintic. Used for menstrual irregularities, contusions and associated painful swelling. Antiseptic for wounds: Crush rhizome and apply to wounds. Externally, rhizomes are applied to insect bites, ringworm, bleeding		hepatoprotective, and anticarcinogenic activity.
7	*Glycyrrhiza glabra*	Licorice	Glycyrrhizin (a triterpenoid saponin), glycyrrhizinic acid, glabin A and B, glycyrrhetol, glabrolie, isoglabrolide, isoflavones coumarins, triterpene sterols	Roots, leaves, and rhizomes	Cultivated. everywhere, Native of southeast Europe and southwest Asia	In China, it is an ingredient in many remedies and used for spasmodic cough. In ancient Greece, China and Egypt, used for gastritis and UGI tract ailments	Mentioned in Ayurveda, Synerveda	Antibacterial, anti-hepatotoxic, estrogenic, antifungal, antihemorrhoidal, antihyperglycemic, antimalarial, antioxidant, antiulcer
8	*Mentha piperita*	Peppermint	Menthol, menthone and menthyl esters, particularly menthyl acetate, limonene, pulegone, caryophyllene and Pi nene	Fresh leaves	India , China, Europe, America, Australia	Nausea, vomiting, abdominal pain, indigestion, irritable bowel, and bloating	Ayurveda, Unani	Antitussive, anti spasmodic , anti emetic, radiprotective, antimicrobial effects
9	*Ocimum sanctum*	Holy Basil, Sulasi	Methyl homo anisic acid, plus cineol and linalool, Eugenol (1-hydroxy-2-methoxy-4-allybenzene)	Rhizomes, leaves	Found throughout the Philippines	Traditionally used for cough, bronchitis, asthma, malaria, dysentery, stress situations, worm infestations, superficial fungal infections, and as diuretic.	Ayurveda, Greek , Roman and Siddha	Antifertility, anticancer, antidiabetic, antifungal, antimicrobial, galactagogue, hepatoprotective, cardioprotective, antiemetic, antispasmodic, analgesic actions.
10	*Piper nigrum*	Black pepper	Piperine, alkamides, piptigrine, wisanine, dipiperamide and dipiperamide	Fruits and roots	Indian sub-continent Vietnam, Burma ,Indonesia	Aid digestion, improve appetite, treat coughs, colds, breathing. Posses Antibacterial activity and reported use in antiprotozoal medicine	Remedies in Ayurveda, Siddha and Unani medicine and also Chinese	Possesses alterative, tonic, appetizer & carminative activities, dyspepsia, flatulence & respiratory tract infection.
11	*Pongamea pinnata*	Karanj, Honge	Glabrin kanugin, gamatay, , glabrosaponin, kaempferol, kanjone, kanugin, karangin, neoglabrin, pinnatin, pongamol, pongapin, quercitin	Fruits and sprout	Coastal regions of India, Australia, Florida, Hawaii, India, Malaysia, Philippines	used in folk remedies for abdominal tumors in India, oil is used as a liniment for rheumatism	Mentioned in Ayurveda	Anti-inflammatory Activity, Anti plasmodial, Anti diarroheal, antiulcer and anti oxidant property
12	*Psidium guava*	Guava	β- caryophyllene, ß-sitosterol, maslinic acid, Pinene	Leav, bark, fruit, roots	Introduced from tropical America. Thoroughly naturalized Pantropic in distribution	In India, water decoction of leaves used for treatment of jaundice, ulcers, rheumatism, wound cleaning and constipation	In Ayurveda it is considered as tridosha nashaka	Antidiarrheal, antiseptic, antispasmodic, antioxidant hepatoprotective, anti-allergy, antimicrobial, antigenotoxic,

								antiplasmodial, cardioactive, anticough, antidiabetic, antiinflammatory, antinociceptive
13	*Punica granatum*	Pomegranate	Ellagic acid ellagitannins (including punicalagins), punicic acid, flavonoids, anthocyanidins, anthocyanins, estrogenic flavonols and flavones.		Native of southwestern Asia. - Has been introduced in all tropical countries	In India, rind of fruit used for diarrhea, In Cuban traditional medicine, used for treatment of respiratory diseases. In traditional Thai medicine, used for diarrhea. Juice of fresh fruit used for dyspepsia and as a cooling and thirst-quenching drink for fevers. The Chinese and Annamites use the rind of the fruit and root bark as vermifuge.	Reported in Ayurveda and Chinese literatures	Anticarcinogenic, anti-inflammatory, antimicrobial properties, with beneficial effects in various disease processes such as Alzheimer's, osteoarthritis, neonatal brain injury, male infertility
14	*Rosemarinus officinalis*	Rosemary	d-pinene, cineol, borneol, camphene and camphor.	Leave	Introduced from Europe. Commonly sold in markets. Cultivated in gardens for medicinal purposes.	Cough, Diuretic, Gas pains, Rheumatism, Conjunctivitis	Mentioned in the Greek folk medicinal system	Antioxidant, radioprotective, Anti inflammatory, Antibacterial, Antihypotensive, Antispasmodic
15	*Sesame spp*		Sesamin, sesamolin, ß-stigmasterol, ß-sitosterol, and stigmasterol-3-O-ß-D-glucoside.	Seeds	Native of tropical Asia	Oil of seed used for treatment of ulcers and suppurating wounds, antirheumatic, Alopecia (baldness) due to prolonged illness, pulmonary tuberculosis	Mentioned in Ayurveda, Unani and Siddha	Oil considered demulcent, emollient, diuretic, emmenagogue, lactagogue and laxative, Antioxidant / Analgesic, neuroprotective, Insecticidal
16	*Syzgium aromaticum*	Clove	Eugenol, acetyl eugenol, beta-caryophyllene and va nillin,crategolic acid	Flower buds	Harvested primarily in Indonesia, India,Madagasc ar, Zanzibar, Pakistan, Sri Lanka	Essential oilis used as an anodyne (painkiller) for dental emergencies, as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis	Indian Ayurvedi c medicine, Chin ese medicine, and western herbalis m	Analgesic, aromatic Antibiotic, antiseptic Anthelmintic, mosquito repellent, anti-rheumatic and carminative agent
17	*Terminalia chebula*	Hardad, Myrobalan	Chebulinic acid, chebulagic acid, chebulic acid, ellagic acid and gallic acid	Fruit	Native to southern Asia from India a nd Nepal east to southwestern China (Yunnan), and south to Sri Lanka, Malaysia and Vietn am.	Decoction of fruit used for thrush and as gargle for mucous membrane inflammations of the mouth, obstinate diarrhea. In India, used for digestive disorders, irregular fevers, flatulence. Used for renal calculi, dysuria, and urinary retentionUsed for fever, cough, and asthma.	Extensively used in Ayurveda, Unani, and Homeopathic medicine.	Antioxidant, antimicrobial, antidiabetic, hepatoprotective, anti-inflammatory, antimutagenic, antiproliferative, radioprotective, cardioprotective, anti-inflammatory, antimutagenic, radioprotective, anticaries, wound healing activity.
18	*Thymus vulgaris*	Green thyme, Garden thyme	Thymol and cavacrol , thujone, pinene, camphene, pinene, cymene, terpinene, , caryophyllene	Sub shrub	It is native in the Mediterranian Europe region	Used earlier for Cold, coughs, asthma, laryngitis, sinusitis, catarrh, whooping cough, sore throats and tonsillitis	Ayurveda ,Greek and ancient Egyptian medicinal folklore	Antiseptic, antibacterial, antispasmodic, astringent, disinfectant

Table 3. Fuzzy Set Membership Analysis for herbal plants screened against *Klebsiella* pneumoniae, on the basis of Weightage Matrix scores

S.No	Herbal Plants	μ_s*	Optimized score
1	*Rosmarinus offcinalis*	1	+++++ (5)
2	*Punica granatum*	0.81	+++++ (5)
3	*Curcuma domestica*	0.9	+++++ (5)
4	*Psidium guajava*	0.73	++++ (4)
5	*Thymus vulgaris*	0.72	++++ (4)
6	*Glycyrrhiza glabra*	0.72	++++ (4)

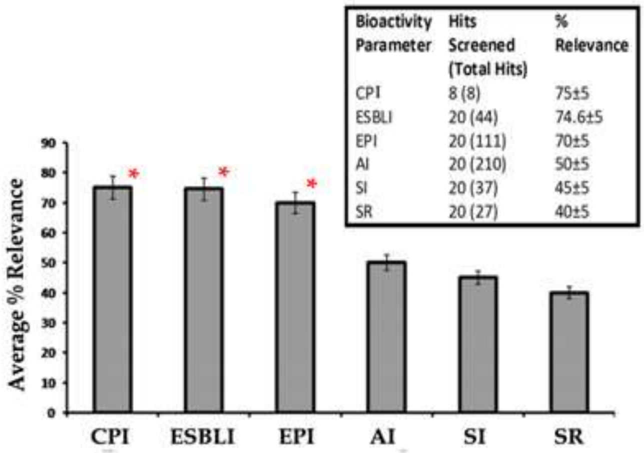

Fig 1: percentage relevance of identified bioactivity parameters using scoring matrix based analysis

******indicates Bioactivity Parameter exhibited significantly (p<0.05) higher % relevance as compared to other parameters i.e. AI, SI & SR*

% Relevance was calculated using the formula:

% (Relevance) avg = (No. of relevant hits based on observational analysis) X 100 / (n=20); (Search engine =PubMed)

CPI: Capsular Polysaccharide Inhibition; ESBLI: Extended Spectrum Beta-Lactamase Inhibition; EPI: Efflux Pump Inhibitor; AI: Adhesion Inhibition; SI: Siderophore Inhibition; SR: Symptomatic Relief

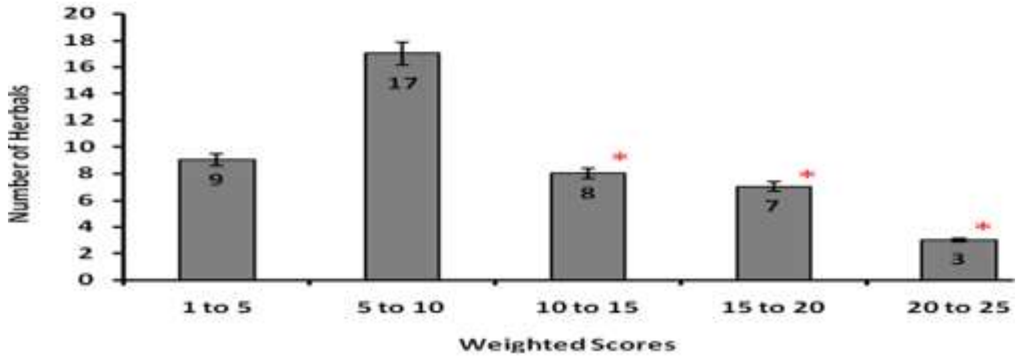

Fig 2 Weightage matrix scores for herbals

Weighted Matrix scores of herbal leads identified using Classical Bioprospection , categorized by median cut off score of 18.5; (a) * Selected herbals (~18) with median score ≥ 18.5 i.e., *Rosmarinus officinalis, Glycyrrhiza glabra, Thymus vulgaris, Psidium guajava, Curcuma domestica, Punica granatum, Ocimum sanctum, Terminalia chebula, Mentha piperita, Brassica nigra, Allium sativum, Syzigium aromaticum, Bridella micrantha, Pongamea pinnata, Artemesinin annua, Camellia sinensis, Piper nigrum, Sesame spp.* (b) Other non selected herbals having a weighted score ≤ 18.5 i.e., *Berberis aristata, Citrus paradise, Nelumbo nucifera, Picorhiza kurroa, Pimpinella anisum, Holoptelea integrifolia, Jatropha elliptica, Daucus carota, Commifora molmol, Calendula officinalis, Eucalyptus globules, Mellisa officinalis, Azadirachta indica, Andrographis paniculata, Dortenia picta, Prospis julifora, Centella asiatica, Coffea arabica, Ricinus communis, Carica papaya, Sinapis alba, Aloe barbadensis, Dasmodium gangeticum, Bergenia crassifolia, Apocynum cannabinum, Plumbago zeylanic*