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In *silico* modeling for Identification of promising antimicrobials of Herbal origin against highly virulent pathogenic strains of bacteria like New Delhi Metallo-beta-lactamase -1 *Escherichia coli*

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ABSTRACT: Drug resistance has mushroomed up after advent of every major class of antimicrobial drugs, varying in time from as short as 1 year (penicillin) to >10 years. Organisms associated with nosocomial and community acquired infections are also becoming drug resistant due to the over utilization of antibiotics, consequently leading to high morbidity, mortality and increased health costs. One such example of a beta lactamase producing bacteria is New Delhi Metallo beta lactamase (NDM-1) producing *Escherichia coli* and *Klebsiella pneumoniae*, and was reported for the first time in the year 2009 in a Swedish patient. NDM-1 bacterial strains possess more potent hydrolysis ability towards almost all antibiotics, including Carbapenems. NDM-1 bacterial strains are sensitive to Tigecycline and Colistin but there is problem of side effects associated with them. In such a situation where NDM-1 strains are displacing antibiotic sensitive strains and are resistant to most of the chemotherapeutic agents, there is a need for the search of novel alternative effective therapeutic agents. The present bioprospective study aims to analyze the potential of various natural plants or their products, based upon bioactivity parameters and presence of chemical constituents, using matrix based modeling, followed by optimization. The outcomes of the lead identification need verification with respect to antimicrobial potential against virulent NDM-1 *Escherichia coli*.

KEYWORDS: Antimicrobial agents, Herbal mitigation, Matrix modeling, Ethno pharmacology, Antibiotic resistance, Superbugs, NDM-1 *Escherichia coli*.

1 INTRODUCTION

Herbal medicine still holds mainstay in about 70% of the world population in primary health care attributed towards its better cultural acceptability, compatibility with the human physiology, possibly lesser side effects and ease of availability in a particular local region [1], [2]. An estimate revealed that about 25% of the drugs prescribed globally are either originated directly from plants or otherwise synthetic derivatives based on plant based precursors [3]. It has been reported that out of WHO recommended 252 basic and essential drugs, nearly 10% are of direct plant origin [4].

India holds 10% of the world's biodiversity, distributed in 16 agro-climatic zones in a peninsular geography, supporting 20,000 medicinal plants, therefore, ranked as one of the 12-megabiodiversity centers of the world [5]. It is revealed that the annual global turnover of herbal medicine industry, in the year 2008, was 90 billion dollars with a limited share of Rs. 2300 Crores to Indian subcontinent [6], [7]. It indicates a huge potential reserve that is yet to be explored for its judicious utilization especially against new world health challenges.

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The evolution of microbes towards antibiotic resistant patterns has been clearly indicated by 4,40,000 new cases of Multi-Drug resistant (MDR) Tuberculosis along with widespread of Extensively Drug Resistant (XDR) Tuberculosis in 64 countries ; 41% cases of Hospital- Acquired MRSA and increased upsurge of Vancomycin Resistant Enterococci (VRE) & Human Immunodeficiency Virus (HIV) infections and/or Gonorrhoea etc. [8], [9], [10]. This has forced the medical practitioners to recommend anti-retroviral and last line cephalosporin antibiotics [11], [12]. NDM-1, a new entrant of 2008, harbored Gram-negative bacilli, present in more than 15 countries has resulted in around 100 deaths till date.

The new officially accepted terminology for NDM-1 associated infections is CRE infections i.e. Carbapenem Resistant Enterobacteriaceae, indicative of a latent infection based alarming situation which might get worsened as it evolves to become resistant against new generation of antibiotics like Polymyxin, Colistin etc. Thus, this potentially dangerous Biothreat agent requires holistic mitigation approach for an effective medical management strategy [13].

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 [14]. 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 [15], [16], [17]. 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.

2 METHODOLOGY

2.1 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.

2.2 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, NDM-1 harbored *Escherichia coli*, the various comparable targets attributing towards bactericidal activity of NDM-1 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 NDM-1 *Escherichia coli*. The seven testing parameters were selected for study based on mechanistic aspects of antibiotic resistance of NDM-1 harboring strains, including Beta lactamase inhibition, presence of phytocompounds, MDR Pump Inhibition, antibiotic action potentiation, quorum sensing inhibition, adhesion inhibition, antibiotic resistance modification and symptomatic relief provision [18]. The rationale supporting selection of these parameters using classical approach for bioprospection studies are given in Table 1.

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

S.No.	Parameter	Rationale for selection (Based on Classical Approach)
1	*β-lactamase inhibition* [19]-[20]	(a) Use of β-lactamase inhibitor with antibiotics as potentiator is an accepted tool to avert resistance, e.g., Augmentin. (b) Several plants have been reported to exhibit β-lactamase inhibition activity and are effective antimicrobial agents, e.g., *Camellia sinensis*, *Papaya carica* etc. (c) β-lactamase, produced by NDM-1 harboring strains, could be targeted to act as an effective bactericidal agent.
2	*MDR Pump Inhibition* [21]-[25]	(a) Multi-Drug Resistant Efflux pumps are bacterial membrane associated active transporters, sustaining the release of drug outside the bacterial cell thereby bestowing antibiotic resistance and restoring the concentration of active drug, e.g., Flavonolignans, Phenothiazines etc. (b) One of the associated phenomenon of resistance offered by NDM-1 *Escherichia coli* is due to the presence of MDR pump namely AcrAB-TolC and AcrEF-TolC classified under RND (Resistance/Nodulation/Division superfamily) which are secondary active transporters driven by ion gradients. (c) Several herbals namely *Aegle marmelos*, *Camellia sinensis*, *Plumbago zeylanica*, *Punica granatum*, *Acorus calamus*, *Hemidesmus indicus*, *Holarrhena antidysentrica*, *Syzygium cuminii* etc., have been shown to contain various molecules like 5'-methoxyhydnocarpin-D, Pheophytin etc., which are reported as MDR pump inhibitors.
3	*Quorum sensing inhibition* [33]-[38]	(a) Quorum sensing is a cell-to-cell signalling mechanism in which bacteria secrete autoinducers, which act as self limiting at a certain threshold concentration by acting at the transcription level and regulating bacterial gene expression. The inhibition of such phenomenon can avert resistance. (b) NDM-1 *Escherichia coli* produce the autoinducer-2 (AI-2), which is synthesized by the product of the luxS gene. Inhibiting the production of AI-2 is a way to control the spread of NDM-1 bacterial infection. (c) *Camellia sinensis*, *Plumbago zeylanica*, *Berberis aristata*, *Hemidesmus indicus*, *Punica granatum*, *Holarrhena antidysenterica*, Horseradish (*Armoracia rusticana)*, Garlic (*Allium sativum*), *Tetrazygia bicolor* (Melastomataceae), *Quercus virginiana* (Fagaceae) etc. are reported to inhibit intracellular communication amongst bacterial populations leading to Quorum sensing inhibition.
4	*Adhesion inhibition* [32]	(a) Major type of adhesion molecules reported include integrins, cadherins, IgCAMS, selectins etc. for which several synthetic agents such as Levocitrizine, Clarithromycin while herbal plants such as *Plumbago zeylanica* and *Aegle marmelos*, possessing Proanthocyanidins, acidic polysaccharide etc. have been reported to be effective in inhibiting the host pathogen interactions. (b) NDM-1 E. coli constitutively release nano-sized outer membrane vesicles (OMVs), Intimin and Adhesin, which could act as the potential targets for therapeutic strategies thereby preventing the adhesion of microorganisms to host cell surfaces.
5	*Antibiotic resistance modification* [29]	(a) Selective pressure induces multi-drug resistant strains, extensive drug resistant strains or pandrug resistant strains, depending on the degree of resistance developed, required to be downgraded. (b) Certain herbal plants have been found to exhibit antibiotic resistance modification activities, e.g., *Rosmarinus officinalis, Caesalpinia spinosa, Camellia sinensis* etc. by the virtue of phytocompounds like Epicatechin gallate, Ethyl gallate, Carnosic acid, Ferruginol, 5-Epipisiferol etc. (c) NDM-1 evolving continuosally, progressing towards a pandemia due to the vulnerability of human physiology and growing selective pressure of antibiotics. This needs to be mitigated at an early stage by the virtue of antibiotic resistance modification phenomenon so as to overcome the probable severe socio-economic impact.
6	*Symptomatic relief provision* [30]-[31]	(a) Palliative care using herbs is an accepted phenomenon that focuses on relieving and preventing the suffering of patients, e.g., *Allium sativum, Allium cepa* and *Ocimum sanctum* for bloating, cold and bronchial congestion respectively. (b) This broadens the scope of study to target less virulent strains of NDM i.e. NDM 2 to 7, with least virulence, however producing similar symptoms. (c) NDM-1 harbored *Escherichia coli* produces symptoms like Urinary Tract infections, Gastroenteritis, Nosocomial Pneumonia, Bacteremia, Wound infection and Fever. Herbal agents are required to prevent the aggravation of infection associated with predefined vulnerability profile of individual.
7	*Presence of phytocompounds* [21], [26]-[29]	(a) Phytocompounds such as flavonoids, tannins, lutein, limonene, theobromine, phytosterols etc. are known to have antibiotic resistance modifying activity, immunostimulation activity and/or bactericidal activity. (b) Holistic approach requires multi-component, multi-targeted, non-specific, immunostimulator and antibiotic resistance modifier herbal plant to be selected as alternative therapeutic modality so as to mitigate lethal impact of NDM-1 associated infections. (c) Selective pressure of Polymyxin and Colistin might lead to new antibiotic resistance pattern in NDM-1, thus holistic mitigation should include diverse phytochemicals enriched herbals as alternative therapeutic substitutes.

2.3 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 + Antimicrobial 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:

$$\textit{Average Percentage Relevance} = \frac{(No.of\ Relevant\ hits\ based\ on\ observational\ analysis * N)}{(n=20)}\ X\ 100$$

Relative weightage for each parameter assigned on the basis of percentage relevance is given in Table 2.

Table 2. Weightage assigned to the parameters based on Average Percentage Relevance

S.No.	Parameter Chosen	Average Percentage relevance (Advanced Search)	Average Percentage relevance (Random Search)	Mean Value	Relative Weightage assigned
1	β lactamase inhibitor	59.4% ± 0.198	19.8% ± 0.140007	39.60% ± 0.171499	5.910
2	Presence of Phytocompounds	44.2% ± 0.138125	16.575% ± 0.097669	30.39% ± 0.119638	4.535
3	MDR pump inhibition	39.25% ± 0.164	6.45% ± 0.115966	22.85% ± 0.14205	3.410
4	Antibiotic action potentiator / enhancer	38.775% ± 0.17625	3.525% ± 0.124628	21.15% ± 0.15266	3.157
5	Quorum sensing inhibition	33.075% ± 0.11025	11.025% ± 0.077959	22.05% ± 0.095494	3.291
6	Adhesion inhibition	20.55% ± 0.0685	6.85% ± 0.048437	13.70% ± 0.059332	2.040
7	Antibiotic resistance modification activity	11.925% ± 0.03975	3.975% ± 0.028107	7.95% ± 0.03443	1.186
8	Symptomatic relief provider	10.05% ± 0.0335	3.35% ± 0.023688	6.70% ± 0.029016	1

2.4 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. The rationale for selected plants is given in Table 3.

Table 3. Selected Herbal plants showing probable utility against NDM-1 Infection [21], [26]

S.No.	Herbal Plant	Ethnopharmacological Importance	Relevance of Herb in Traditional Medicine	Availability	Vedic Literature supporting its use	Prior investigation	Indirect Indications (if any)
1							Symptomatic relief in case of *E. coli* associated infections
2							Antibiotic action enhancement with Tetracycline and ciprofloxacin
3	*Allium sativum*	-	Used in bronchitis, constipation, joint pain and fever	Central to southern Asia	Recorded from Vedic times, from ancient Chinese to Egyptian culture	Alliin, the active compound, is antimicrobial, lipid-reducing, antioxidative and fibrinolytic	Antibacterial, antimycotic and Lipid-reducing effects also reported
4							Produce MDR inhibitors i.e. pheophorbide and methoxyhydranocarpin, hence may act as antibiotic resistance modifier
5							-
6	*Hemidesmus indicus*	Aphrodisiac, antipyretic, alexiteric, antidiarrhoeal, astringent to bowels and useful in treatment of fevers, foul body odour, asthma, bronchitis, blood disorders, leucorrhoea, dysentery, diarrhoea, thirst, burning sensation, piles, eye troubles, epileptic fits, poisoning, rat bites etc	Diaphoretic, diuretic and blood purifier	Found from the upper Gangetic plain eastwards to Assam in India	Reported in Ayurveda and Unani Medicine System	Employed in nutritional disorders, syphilis, chronic rheumatism, gravel and other urinary diseases	-

7							-
8	*Papaya carica*	Medicine for dyspepsia, hyperacidity, dysentery and constipation	Antihelminthic and amoebicidal activity, blood pressure reducing agent	Suitable for tropical climate of India	Reported in Ayurveda	Seeds - antibacterial properties	Effective against *E.coli, Salmonella* and *Staphylococcus* infections
9							-
10	*Punica granatum*	Used to treat diarrhea, dysentery, vomiting and eye pain. Also in hemorrhoids and as a gargle in cases of sore throat	Tapeworm infestation, diarrhea and dysentery, as an abortifacient and astringent	Probably originated in Asia	Reported in Ayurveda and Chinese literatures	Used for gastrointestinal disturbances and bacterial infections	Antibiotic action enhancement with Chloramphenicol, Gentamicin, ampicillin, Tetracycline and oxacillin
11	*Syzygium cuminii*	Seed – Antidiabetic, atonic and spastic constipation, Pancreatic complaints, nervous disorders and as a diuretic	Bronchitis, asthma, and dysentery, Also used for ulcers, leucorrhea, Stomachache, fever, dysuria, and inflammation	Indigenous to the east Indian Malaysian Region	Reported in Ayurveda and Unani Medicine System	Proved to be effective against Diarrhea Inflammation of the mouth, skin and pharynx	-

2.5 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 8 for any plant. The cut off value selected for this matrix based analysis is closest value to the median of 1-8 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.

2.6 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 Step No. 2.5 [39]. 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 [40]. This step identifies potential plant leads based on *in silico* bioprospection approach subjected to fuzzy set membership analysis and optimization to validate the findings. Weightage matrix score for the selected herbal plants is exemplified in Table 4.

Table 4. Weightage Matrix Scores for herbal plants screened on the basis of binary matrix scores (Scores > 3)

Character weightage	Weightage: 5.910	Weightage: 4.535	Weightage: 3.410	Weightage: 3.157	Weightage: 3.291	Weightage: 2.040	Weightage: 1.186	Weightage: 1	
Herbal Plant	**β-lactamase inhibitor**	**Presence of phyto-compounds against NDM-1**	**MDR Pump inhibition**	**Antibiotic action potentiator / enhancer**	**Quorum sensing inhibition**	**Adhesion inhibition**	**Antibiotic resistance modification activity**	**Symptomatic relief provider**	**Total**
Aegle marmelos	-	-	+	+	-	+	+	-	**9.793**
Camellia sinensis	+	-	+	+	+	-	-	-	**15.768**
Plumbago zeylanica	-	+	+	-	+	+	-	-	**13.276**
Berberis aristata	-	+	-	+	+	-	-	-	**10.983**
Punica granatum	-	-	+	-	+	+	-	-	**8.741**
Rosmarinus officinalis	-	+	-	-	+	-	+	-	**9.012**
Acorus calamus	-	-	+	+	+	-	-	-	**9.858**
Hemidesmus indicus	-	-	+	+	+	-	-	-	**9.858**
Holarrhena antidysentrica	-	-	+	+	+	-	-	-	**9.858**

2.7 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 [41]-[42]. Scores after fuzzy set membership analysis of selected herbal plants are represented in Table 5.

Table 5. Fuzzy Set Membership Analysis for herbal plants screened on the basis of Weightage Matrix scores

S.No.	*Herbal Plant*	*μS**	*Optimized Score*
1	*Camellia sinensis*	1	+++++++ (7)
2	*Aegle marmelos*	0.5625	+++++ (5)
3	*Plumbago zeylanica*	0.8125	+++++ (5)
4	*Berberis aristata*	0.5625	++++ (4)
5	*Punica granatum*	0.375	+++ (3)
6	*Rosmarinus officinalis*	0.375	+++ (3)
7	*Acorus calamus*	0.1875	+++ (3)
8	*Hemidesmus indicus*	0.1875	+++ (3)
9	*Holarrhena antidysentrica*	0.0625	+++ (3)

** μS = [(S) – min(S)] / max(S) – min(S)], where [S] is the Weightage matrix score*

2.8 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.

3 RESULTS

3.1 KEYWORDS HITS SCORING MATRIX

On the basis of the keyword hits scoring results weightage was given to various parameters selected for screening of herbal plants with respect to antimicrobial activity. Weightage was decided according to the percentage relevance obtained

for each parameter, as elucidated in table 2. Highest percentage relevance was obtained for β-lactamase inhibitor, followed by other parameters like Presence of phyto-compounds against NDM-1, MDR Pump inhibition, Antibiotic action potentiator / enhancer, Quorum sensing inhibition, Adhesion inhibition, Antibiotic resistance modification activity and Symptomatic relief provider. Consequently weightage factors were given to selected parameters in the range of 1-6, based on statistical unitary approach, with highest weightage i.e. 5.910, given to β-lactamase inhibitor, followed by other parameters in decreasing order, as explicated in Table 4.

3.2 BINARY (PRESENCE-ABSENCE) COEFFICIENTS MATRIX

Out of 50 herbal plants, 9 herbal plants were shown to contain either 3 or more than 3 characteristic and hence illustrated a better score as compared to other herbs, e.g. *Aegle marmelos, Acorus calamus, Berberis aristata, Camellia sinensis, Hemidesmus indicus, Holarrhena antidysentrica, Punica granatum, Plumbago zeylanica* and *Rosmarinus officinalis*, as shown in figure 1. Also 11 herbal plants although had low scores but exhibited specific bioactivity related features fulfilling the criteria to be chosen as a therapeutic aid against multidrug resistant bacteria such as the presence of MDR efflux pumps and β lactamase inhibition potential, e.g. *Allium neapolitanum* (Spring Garlic), *Borago officinalis* (Gaojaban), *Papaya carica* (Papita), *Piper longum* (Long pepper), *Pinus nigra* (Pine), *Rheum officinalis* (Rhubharb), *Ranunculus repens* (Crowfoot), *Sinapis alba* (White Mustard), *Spartium junceum* (Spanish broom), *Senebierra didyma* (Senebierra) and *Spondias mombin* (Taperiba).

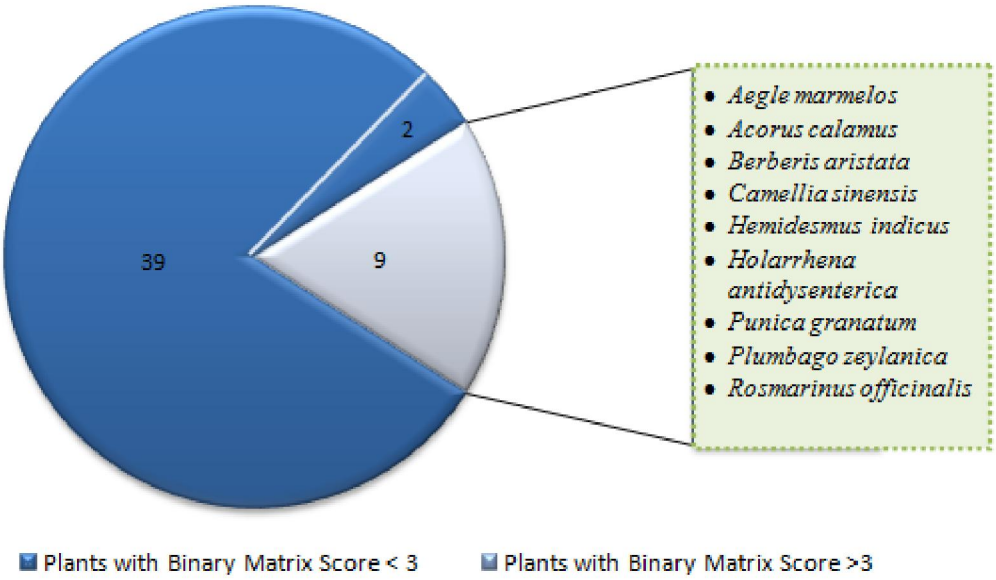

Fig. 1. Binary Matrix Scores for Herbal Plants

Out of the 50 Plants studied, 39 Plants had a Binary matrix score of 1 (Abrus precatorius, Allium neapolitanum, Andrographis paniculata, Azadirachta indica, Adhatoda vasica, Beta vulgaris, Borago officinalis, Capsicum, Camomile, Casuarina equistifolia, Caesalpinia spinosa, Coffea, Caesalpinia bonducella, Cyperus rotundus, Daucus carota, Euphorbia hirta, Ficus religiosa, Jatropha elliptica, Lawsonia inermis, Nelumbo nucifera, Ocimum sanctum, Papaya carica, Plantago ovata, Piper longum, Pinus nigra, Polyathia memorials, Rheum officinalis, Ranunculus repens, Sinapis alba, Spartium junceum, Senebierra didyma, Spondias mombin, St. John's wort, Tagetes, Terminalia arjuna, Terminalia belerica, Thymus vulgaris, Vitis vinifera and Ziziphus jujube) ; 2 plants had a Binary Matrix Score of 2 (Allium sativum and Syzygium cuminii) and rest 9 plants had a binary matrix score of ≥ 3 (Aegle marmelos, Acorus calamus, Berberis aristata, Camellia sinensis, Hemidesmus indicus, Holarrhena antidysentrica, Punica granatum, Plumbago zeylanica and Rosmarinus officinalis).

3.3 SIMPLE ADDITIVE WEIGHING MATRIX

Out of 9 plants selected on the basis of binary coefficient matrix (Binary Matrix score ≥ 3), it was revealed that 3 herbal plants showed immense potential of acting as a therapeutic agent against drug resistant microorganisms, as their combined weightage scores were even higher than the median value score i.e. 9.858, e.g. *Camellia sinensis, Plumbago zeylanica* and *Berberis aristata* as shown in Table 4.

3.4 DECISION MATRIX

On the basis of Decision matrix, 3 plants were found to show high percentage relevance to be chosen as potent therapeutic herbal plants against drug resistant bacteria, as shown in Table 5. Amongst these, *Camellia sinensis* (Green Tea) held the topmost position with 100% relevance, followed by *Plumbago zeylanica* (Chitraka), *Aegle marmelos* (Bel), *Berberis aristata* (Daruhaldi), *Punica granatum* (Anar), *Rosmarinus officinalis* (Rosemary) and others.

3.5 OPTIMIZED SCORING

Optimized values were given to the selected herbal plants, on the basis of which top 3 relevant herbal plants were revealed, e.g. *Camellia sinensis, Plumbago zeylanica* and *Berberis aristata*, as shown in Table 5.

4 DISCUSSION

Antibiotics have become mainstay of our lifestyle disease control strategy as these chemotherapeutic agents are often used to treat many infectious diseases. An overuse and abuse of such agents has led to the transformation of the antibiotic sensitive bacterial strains into resistant ones through natural selection and defined mutations. An alternative pipeline of herbal/natural therapeutic agents have to be searched for the adequate control of the emergence, re-emergence and spread of antibiotic resistant microorganisms like NDM-1 *Escherichia coli*, Methicillin Resistant *Staphylococcus aureus*, Multi-Drug Resistant Tuberculosis.

The present study is an attempt to combine classical literature based analysis with statistical interpretation of data output obtained by both random and advanced search model using the PubMed web tool. The seven parameters are selected on the basis of their direct significance in a) symptomatic relief provision, b) antibiotic resistance modification, c) antibiotic action potentiation, d) drug efflux inhibition (Table-1). Similar study using web tools for bioprospection has been done by Elizabeth S. Jenuwine *et al* for the evaluation of sleep wake cycle in healthy individuals (selected parameter) [43]. This similar model using multi-parametric approach has been used in conjunction with evaluation of their relative relevance based on priority indexing (Table-2)

The classical bioprospection of natural plant products has provided drugs of immense importance e.g., Taxol (anticancerous, *Taxus baccata*), Quinine (Antimalarial, antipyretic, *Cinchona officinalis spp.*) Vincristine (anticancerous, *Rosmarinus officinalis)*, Vinblastin (anticancerous, *Rosmarinus officinalis)*. In this study, selection of plants has been done on the basis of parameters like ethnopharmacological importance, use in traditional medicine, ease of availability, any indication in Vedic literature or available scientific evidence for complimentary use. Such investigatory analysis has provided 50 plants showing variable significance with respect to different descriptors chosen.

The plants were subjected o both random and advanced search model, (Using PubMed as search engine) followed by Binary Coefficient matrix analysis ($p < 0.05$), revealing 11 out of 50 plants to be used for *in silico* cross matrix bioprospection analysis. Binary coefficient matrix analysis is used to extract items of choice with probable higher significance based on all or none principle. This practice removes bulk outliers, thereby reduces database size to measurable proportion. This is achieved by selecting plants with binary score > 3, so as to scale down the number of plants from 11 to 9 and thereby reducing the timeframe of screening significantly (Figure 1).

The selected 9 plants are subjected to *in silico* bioprospection model where additive weightage matrix based analysis is performed to analyze the weighted scores for each selected herbal plant on the basis of presence of each weighted parameter. This matrix works on two principles a) binary (0/1) presence or absence law and b) weightage scoring analysis in conjunction. The net scoring was analysed in priority ranking providing relevance of natural plants with respect to their possible role in overall antimicrobial activity against NDM-1 *Escherichia coli* (Table 4). The optimization of obtained data was a fuzzy set membership analysis for decision matrix, revealing 04 plants with optimization score > 3.

5 CONCLUSION

As resistance to old antibiotics spreads, the development of new antimicrobial agents has to be advanced if the problem is to be contained. Bio-assay would reveal the presence of multiple antimicrobial compounds or synergic effects of these compounds. Therefore, standardization of active fractions and study for toxicity and in vivo efficacy may result in development of better antimicrobial drugs. It may provide nature friendly and cheap drugs accessible to all the people of world.

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REFERENCES

[1] Kamboj V.P., "Herbal medicine," *Current Science*, vol. 78, pp. 35-39, 2000.
[2] Weiss R.F., Fintelmann V., *Herbal Medicine*, 2nd English edition, New York: Thieme, 2000.
[3] Inamdar N., Edalat S., Kotwal V.B., Pawar S., "Herbal drugs in milieu of modern drugs," *International Journal of Green Pharmacy*, vol. 2, pp. 2-8, 2008.
[4] World Health Organization, *WHO Global atlas of traditional, complementary and alternative medicine*, Geneva, 2005.
[5] Raskin I., David M. Ribnicky, Komarnytsky S., Ilic N. et al., "Plants and human health in the twenty-first century," *TRENDS in Biotechnology*, vol. 20, no. 12, pp. 522-531, 2002.
[6] Navdeep S., Koyal S., Singh G.K., "Establishment of an Herbal Industry in India," *International Journal of Pharmaceutical & Biological Archives*, vol. 2, no. 4, pp.1011-1019, 2011.
[7] Agrawal O.P., Raju P.S., Abdin M.Z., Abroi Y.P., *Global market of herbal products: Opportunities for India, Traditional system of medicine*, Narosa Publishing House: New Delhi, India; pp. 5-10, 2006.
[8] Giovanni B.O., Marco F., Mario V., "Surveillance and Management of Multidrug-resistant Microorganisms," *Expert Rev Anti Infect Ther,* vol. 9, no. 8, pp. 653-679, 2011.
[9] WHO Fact Sheet on Antimicrobial Resistance, 2012.
[10] CDC Fact Sheet, 2013.
[11] Shahcheraghi F., Nobari S., Rahmati Ghezelgeh F., Nasiri S., Owlia P., Nikbin V.S., Imani Fooladi A.A., "First report of New Delhi metallo-beta-lactamase-1-producing Klebsiella pneumoniae in Iran," *Microb Drug Resist,* vol. 9, no. 1, pp. 30-6, 2013.
[12] Chauhan A., "New Delhi Metallo-beta-lactamase: A misnomer?" *International Journal of Clinical Cases and Investigations*, Vol. 1, no. 5, pp. 5-6, 2010.
[13] Schechner V., Kotlovsky T., Tarabeia J., et al., "Predictors of rectal carriage of carbapenem-resistant Enterobacteriaceae (CRE) among patients with known CRE carriage at their next hospital encounter," *Infect Control Hosp Epidemiol*, vol. 32, no. 5, pp. 497-503, 2011.
[14] Mary J. A., Priyadharshini K.C., Amal G.P.R., et al., "MEDDB: A medicinal plant database developed with the information gathered from tribal people in and around Madurai, Tamil Nadu," *Bioinformation*, vol. 8, no. 8, pp. 391–393, 2012.
[15] Tao Qin, Tie-Yan Liu, Xu-Dong Zhang, Zheng Chen, Wei-Ying Ma, "A Study of Relevance Propagation for Web Search," *International Conference on Research and Development in Information Retrieval* (SIGIR), 2005.
[16] Baeza-Yates R., Ribeiro-Neto B., *Modern Information Retrieval*, Addison Wesley, 1999.
[17] Robertson S. E., and Sparck Jones K., "Relevance Weighting of Search Terms," *Journal of the American Society of Information Science*, vol. 27, no. May-June, pp. 129-146, 1976.
[18] Thakur P., Chawla R., Chowdhry A., Chakotiya A., Singh N., Goel R., Kaushik V., Arora R., Sharma R.K., "Targeting Antibiotic Resistant Bacteria NDM-1 *Escherichia coli* adopting Ethnopharmacological Approach: An alternative strategy to counteract superbugs menace," *Journal of Antimicrobials Photon,* vol. 128, pp. 147-156, 2013.
[19] Beta-Lactamase Inhibitors, *Department of Nursing of the Fort Hays State, University College of Health and Life Sciences*, October 2000.
[20] Beta-Lactamases in laboratory and clinical resistance, *Clinical Microbiology Review*.
[21] Ahmad I., Farrukh A. and Mohd. Owais, "Modern Phytomedicine: Turning Medicinal Plants into Drugs," eds. 15, pp. 199-226.
[22] Webber M.A., Piddock L.V.J., "The importance of efflux pumps in antibiotic resistance," *Journal of Antimicrobial Chemotherapy*, vol. 51, pp. 9-11, 2003.
[23] Li H.Z., Nikaido H., "Efflux-mediated drug resistance in bacteria," *Drugs*, vol. 64, pp. 159-204, 2004.
[24] Piddock L.V., "Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria," *Clinical Microbiology Review,* vol. 19, pp. 382-402, 2006.
[25] Stermitz F.R, Beeson T.D., Mueller P.J., Hsiang J., Lewis K., "Staphylococcus aureus MDR efflux pump inhibitors from a Berberis and a Mahonia *(Sensu strictu)* species," *Biochemical Systematics and Ecology*, vol. 29, no. 8, pp. 793-798, 2001.
[26] Alviano D.S., Alviano C.S., "Plant extracts: search for new alternatives to treat microbial diseases," *Current Pharmaceutical Biotechnology*, vol. 10, no.1, pp. 106-21, 2009.
[27] Gibbons S., "Anti-staphylococcal plant natural products," *Nat. Prod. Rep*., vol. 21, pp. 263-277, 2004.

[28] Gibbons S., Moser E., Kaatz G.W., "Catechin gallates inhibit multidrug resistance (MDR) in Staphylococcus aureus," *Planta Med*., vol. 70, no. 12, pp. 1240-1242, 2004.
[29] Gibbons S., Oluwatuyi M., Veitch N.C., Gray A.I., "Bacterial resistance modifying agents from Lycopus europaeus," *Phytochem*., vol. 62, no. 1, pp. 83-87, 2003.
[30] Hemaiswarya S., Kruthiventi A.K., Doble M., "Synergism between natural products and antibiotics against infectious diseases", *Phytomedicine*, vol. 15, pp. 639–652, 2008.
[31] Clement Y.N., Morton-Gittens J., Basdeo L., Blades A., Francis M.J., Gomes N., Janjua M., Singh A., "Perceived efficacy of herbal remedies by users accessing primary healthcare in Trinidad," *BMC Complementary and Alternative Medicine*, vol. 7, pp. 7-4, 2007.
[32] Woo J. S., Ha B. H., Kim T. G., Lim Y., Kim K. H., "Inhibition of Helicobacter pylori adhesion by acidic polysaccharide isolated from *Artemisia capillaris*," *J. Microbiol. Biotechnol*., vol. 13, pp. 853-858, 2003.
[33] Diggle S., Crusz S., Cámara M., "Quorum sensing," *Current Biology*, vol. 17, no. 21, 2007.
[34] Jakobsen T.H., Bragason S.K., Phipps R.K., Christensen L.D. et al., "Food as a source for QS inhibitors: Iberin from Horseradish Revealed as a Quorum Sensing Inhibitor of Pseudomonas aeruginosa", Department of International Health, Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen, Denmark.
[35] Smyth A.R., Cifelli P.M., Ortori C.A., Righetti K., Lewis S., Erskine P., Holland E.D., Givskov M., Williams P., Cámara M., Barrett D.A., Knox A., "Garlic as an inhibitor of Pseudomonas aeruginosa quorum sensing in cystic fibrosis--a pilot randomized controlled trial", Division of Child Health, University of Nottingham, Nottingham, UK.
[36] Choo J.H., Rukayadi Y., Hwang J.K., "Inhibition of bacterial quorum sensing by vanilla extract", Department of Biotechnology, Yonsei University, Seoul, Republic of Korea.
[37] Rasmussen T.B., Bjarnsholt T., Skindersoe ME.., Hentzer M., Kristoffersen P., Köte M., Nielsen J., Eberl L., Givskov M., "Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector", Center for Biomedical Microbiology, BioCentrum-DTU, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark.
[38] Adonizio A.L., Downum K., Bennett B.C., Mathee K., "Anti-quorum sensing activity of medicinal plants in southern Florida", Dep*artment of Biological Sciences, Center for Ethnobiology and Natural Products, CENaP, Florida International University,* USA.
[39] Cheetham Alan H. and Hazel Joseph E., "Binary (Presence-Absence) Similarity Coefficients", *Journal of Paleontology*, vol. 43, no. 5, pp. 1130-1136, 1969.
[40] Yager R., "On ordered weighted averaging aggregation operators in multi-criteria decision making, IEEE Transactions on Systems," *Man, and Cybernetics*, vol. 18, pp. 183-190, 1988.
[41] Meimandipour A., Hosseini Said A., Lotfolahian H., Hosseini Said J., Hosseini Said H., Sadeghipanah H., "Multiattribute decision-making: use of scoring methods to compare the performance of laying hen fed with different levels of yeast", *Italian Journal of Animal Science*, vol. 11, no. 15, 2012.
[42] Triantaphyllou E., Shu B., Nieto Sanchez S., and Ray T., "Multi-Criteria Decision Making: An Operations Research Approach", *Encyclopedia of Electrical and Electronics Engineering,* vol. 15, pp. 175-186, 1998.
[43] Jenuwine Elizabeth S., "Comparison of Medical Subject Headings and text-word searches in MEDLINE to retrieve studies on sleep in healthy individuals," *J Med Libr Assoc*., vol. 92, no. 3, pp. 349–354, 2004.