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ORIGINAL ARTICLE **OPEN ACCESS**

Bioprospection study to identify herbals targeting opportunistic infection *Neisseria gonorrhoeae*

Navjot Rana[1], Rashmi Wardhan[4*], Ruby Sharma[6], Ayesha Ali Zaidi[2], Raman Chawla[1], Rajesh Arora[3], Haider Ali Khan[3], Ankit Tanwar[5*]

[1]Division of CBRN Defence, Institute of Nuclear Medicine and Allied Sciences, Delhi - 110054, India
[2]Department of Medical Elementology & Toxicology, Jamia Hamdard, Delhi - 110062, India
[3]Department of Biochemical Sciences (DBCS), Defence Institute of Physiology and Allied Sciences (DIPAS), Delhi, India
[4]Department of Biochemistry, Shivaji College, University of Delhi (DU), New Delhi-110027, India
[5]Department of Cell Biology, Albert Einstein College of Medicine, New York, NY, 10461, USA
[6]Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY, 10461, USA
*Corresponding author's Email: *tanwar.ankit9@gmail.com, *rashmiwardhan56@gmail.com

ABSTRACT

Neisseria gonorrhoeae is an infectious gram-negative diplococci bacterium, which is transmitted through sexual routes and mainly affects the reproductive system causing Sexually Transmitted Diseases (STDs) like gonorrhoea. This disease can also be passed from mother to child. Gonorrhoeae is the second most prevalent and commonly communicable disease in the United States. Inflammatory mediators such as Porin, Transferrin binding protein, Hemoglobin binding protein, IgA1 protease, and Penicillinase beta-lactamase are known to play an important role in the pathogenesis of this disease and have been considered as virulence factors. Various research is being conducted to target these molecules, to prevent it, however, this disease is still a challenge due to the various side effects associated with the synthetic drugs. To combat this problem, medicinal plants and their macromolecules are being considered feasible. In the present study, we have utilized an herbal in-silico bioinformatics model to develop a natural remedy with few or no side effects and which can inhibit these virulence factors of this disease. A bio-prospection model was used where the random search was done, which was followed by a rationale-based selection of natural plant products targeting the Gonorrhoeal factors. The model conducted provided with Actocarpus heterophyllus, Cinnamomum zeylanicum, Oxalis corniculata, Rosa indica L., Argyreia nervosa, Abrus precatorius, Holarrhena antidysenterica L., Lantana camara, Piper longum L., Thymus vulgaris, and Zingiber officinale rosc. These plants can be further studied at the in-vitro or in-vivo level to combat this disease.

KEYWORDS*- Neisseria gonorrhoeae, Gram-negative bacteria, Gonorrhoeae, Sexually Transmitted Disease, Herbal informatics.*

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INTRODUCTION

Nosocomial infections occur universally and influence both the established as well as resource-poor countries. In the United States, the Centre for Disease Control and Prevention assessed approximately 1.7 million hospital associate infections, from all types of microorganisms, including bacteria, combined cause contribute to 99,000 deaths each year. (1) *N. gonorrhea* is the causative agent of gonorrhoea, a sexually transmitted disease that causes infections in reproductive organs**.** The organism also retorts as *an* opportunistic infection in many inflammatory disorders like arthritis, cancer, diabetes.

N. gonorrhoeae, become resistant to available antibiotics would greatly complicate the treatment of gonorrhoea (2). Previously recommended antimicrobials cannot again be routinely prescribed for empiric gonorrhea treatment as it also maintains previously acquired antimicrobial resistance phenotypes, regardless of the possibility that the antimicrobial is no more utilized for treatment. In 2011, 11.8% of isolates were penicillin-resistant, 22.7% were tetracycline-resistant, and 13.3% were fluoroquinolone-resistant. Unlike resistance mutations in many other bacteria, resistance mutations in *N. gonorrhea* might improve the survival of resistant strains, even in the absence of antimicrobials.

New antimicrobial treatment selections are needed, but in the long-term goal for using synthetic compounds it will become resistant to that antibiotic or it causes side effects (3). Although the history of

herbal medicine provides decades, sometimes centuries, of anecdotal information, scientific study of herbal medicine is relatively new with low side effects as compared to synthetic drugs (4).
Worldwide business turnover of natural medications is around 62.0 billion dollars with 2% sparing offer of India took after by 45% offer of a European country, 11% of North America, 16% of Japan, 19% of Asian nation and 4.1% of China (5). India shows a positive sign for the worldwide home-grown markets as it has 16 agro-climatic zones, 10 vegetation zones, 15 biotic provinces, 426 biomes, 45000 distinctive plant species, and 15000 therapeutic plants. In addition it includes customary pharmaceuticals like 7000 in Ayurveda, 700 in Unani, 600 in Siddha medications and 30 in other advanced drugs, World Health Organization, WHO Global map book of conventional, correlative and option prescription. Later, this will make India a standout amongst the most 12 mega various nations of the world, which reveals the importance of natural compounds among the various countries for their therapeutic goal.
The present study includes utilizing *in silico* herbal bioprospection modeling, literature-based parameter selection, priority indexing using the random search model, scoring, and decision matrix-based analysis followed by optimization and validation. Such tools can be used to validate findings of classical bioprospection. This study has provided an insight into systematic collection and analysis of literary data to obtain a logical output for ascertaining a desired biological activity. (6)

MATERIAL AND METHODS

Classical literature surge model

The microorganism was selected as it has continuously decreasing treatment regimens and high spread behaviour(7). A classical literature surge model was utilized and ascertained 05 virulence factors like *Porin, Transferrin binding protein, Hemoglobin binding protein, IgA1 protease, Penicillinase beta-lactamase* as virulence factor shown in Table 1. Similarly, the database set of herbals was selected with attributable factors as a) Ethno-pharmacological 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 of the potential of the herb; f) Indirect indications if any. (8)

Relevance Factor -Binary Matrix analysis

The combination keyword search converging antimicrobial activity and virulence factor followed by linking it with observation-based analysis of first 20 hits, relevance factor / net weightage of each virulence factor was evaluated using the following formula:

$$\%\,(Relevance)avg = \frac{(No.\ of\ relevant\ hits\ based\ on\ observational\ analysis)}{Total\ No.of\ Hits\ screened} X\ 100$$

The combination of primary database set of NPPs concerning presence/absence of relevance by PubMed search engine (n= first 20 hits) against 'Bioactivity Parameter + Selected Plant' random search model was tested using Binary Matrix analysis with median value = 03 as cut off filter value. (9)

Relevance Factor linked weightage matrix-based analysis

The relevance factor of each virulence factor was used to define the weightage of every plant with all virulence factors taken together to enhance the 'uncertainty factor' required for statistically valuable outcome by reducing the investigator's biasness and plants with scores ≥8 from the previous step were only utilized to identify potent leads.

Fuzzy set membership analysis for decision matrix & optimization

The mathematical relationship was used to ascertain relative relevance within an identified set of herbals

$$\mu S = \frac{(S - Min\ S)}{(Max\ S - Min\ S)}$$

Where: vs. represents the desirability values of selected NPPs of the fuzzy set S. Min (S) and max (S) are minimum and maximum scores, respectively, in the fuzzy set S. The estimated μS were converted into a leveled score by using a scaled magnitude as optimization of identifying the potential set of NPPs (10).

RESULTS

Classical Literature Surge

The classical literature surge revealed 05 virulence factors **(Table-2)** and herbals with relevance towards 05 efficacy-linked descriptors as exemplified in **Table-3.**

B. Relevance hits scoring

The keyword hits, scoring analysis, % relevance factor was estimated for all the 05 virulence factors identified using classical literature surge. The highest percentage relevance was obtained *Hemoglobin binding protein inhibition* followed by other parameters like to ascertain *Porin inhibition, Transferrin binding protein inhibition, IgA1 protease inhibition, Penicillinase beta-lactamase inhibition*. Consequently, weightage factors were given to selected parameters in the range of 0.05-5 based on the statistical unitary approach **(Table 4; Figure 1)**

C.Simple additive weightage matrix

Out of 50 herbal plants, 27 plants were selected based on the binary coefficient matrix (Binary Matrix score ≥ 4), it was revealed that 26 herbal plants showed the immense potential of acting as a therapeutic agent against drug-resistant microorganisms, as their combined weightage scores were significantly higher than the median value score i.e.12.5, e.g. *Pinus roxburghii, Pterocarpus santalinus, Rosa indica L., Sida cordifolia, Tephrosia purpurea, Celosia cristata, Ficus racemosa, Leptadenia reticulata, Vitex negundo, Argyreia nervosa, Abrus precatorius, Ficus benghalensis, Holarrhena antidysenterica L., Lantana camara, Nymphaea lotus, Abrus precatorious, etc.* as exemplified in Figure 2.

Decision matrix-based Optimization

Based on decision matrix analysis, 12 herbal 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, herbals with a μS score being 1 include *Actocarpus heterophyllus, Cinnamomum zeylanicum, Oxalis corniculata, Rosa indica L., Argyreia nervosa, Abrus precatorius, Hoiarrhena antidysenterica L., Lantana camara, Piper longum L., Thymus vulgaris, and Zingiber officinale rosc.* Optimized scores were also obtained for the selected plants based on fuzzy set membership analysis

Table -1 Rationale for selection of bioactivity parameters

S. No	Bioactivity Parameter	Rationale For Selection (Based on classical approach)
1.	**IgA1 protease**	a) Interferes with the barrier functions of mucosal IgA antibodies by cleaving secretory IgA1 in the hinge region, Cleavage of IgA1 antibodies leads to separation of the F (ab) fragment, which is involved in antigen binding, from the Fc domain, which is involved in effectors function. b) *Rose petal, Ashwagandha, celosia cristata* are involved in antigen-binding activity.
2.	**Iron uptake** (HmbR)	a) Responsible for binding hemoglobin with a high affinity, stripping heme from hemoglobin, and transporting only the heme into the periplasm. Hemoglobin utilization mediated by HmbR is a TonB-dependent process and the utilization of iron bound to transferrin or iron chelates. b) *Achillea millefolium, Ashwagandha, Boerhavia diffusa* are responsible for binding hemoglobin with high affinity.
3.	**β-lactamase**	a) Penicillinase is a specific type of β-lactamase, showing specificity for penicillins, again by hy drolyzing the β-Lactam ring. It deactivates penicillins by destroying the beta-lactam ring via hydrolysis. Beta-lactamase allows bacteria to be resistant to penicillin. b) *Pterocarpus santalinus, Ficus racemosa, Leptadenia reticulata* hydrolyzed the beta lactam ring and destroy the pathogen.
4.	**Porins**	a) *N. species* produces two Porins, PorA and PorB, N. gonorrhoeae expresses only one porin, P or B, translocate spontaneously as functional voltage gated ion channels into plasma membranes of eukaryotic cells, causing a transient change in membrane potential and interference with cell signaling. b) *Artocarpus heterophyllus, Phyllanthus niruri* .are responsible for the process of cell signaling
5.	**Tbp (Transferrin-binding protein)**	a) The tbp locus is a bicistronic operon consisting of tbpA and tbpB. Unlike the case for many other genes of Neisseria, there is no phase variation of the transferrin receptor. TbpA is the TonB-dependent transferrin receptor and Fe transport channel, which forms complex with TbpB. TbpB is the transferrin receptor accessory lipoprotein b) *Oxalis corniculata, Pinus roxburghii, Raphanus sativus* play a major role in transferring protein.

Table 2- Percentage relevance of identified bioactivity parameters using scoring matrix-based analysis

S.No.	Bioactivity parameters	Total Hits Observed	Hits relevant (n=20)	Mean % Relevance	Relative weightage assigned
1.	Porin inhibition	196	5	25	1.44
2.	Transferrin binding protein inhibition	421	3	15	1.15
3.	Hemoglobin binding protein inhibition	251	6	31.66	5
4.	Penicillinase beta-lactamase inhibition	135	5	25	1.44
5.	IgA1 protease inhibition	650	4	21.66	2.88

Table -3: Rationale for selection of herbal compounds from ethnopharmacological importance

S.No.	Herbal Plant	Common name	Parts utilized	Availability	Relevance of Herb in Traditional Medicine	Relevance in Vedic Literature	Current Indications
1	*Artocarpus heterophyllus*	Jack Fruit,	Seeds, Jack Fruit, Leaves	Native to the southern Medi terranean regi on 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.	Siddha Unani	Leaves are useful in fever, boils, wounds, skin diseases. The young fruits are acrid, astringent, carminative, and tonic.
2	*Argyreia nervosa*	Hawaiian Baby Wood rose Seeds	Seeds, Wood	India.	Acrid, bitter, astringent, sweet, emollient	Ayurveda Siddha Unani	The roots are acrid, bitter, astringent, sweet, and emollient. It is used in vitiated conditions kapha and vita, emaciation, wounds, ulcers, anorexia, dyspepsia, flatulence. It is widely used for Psychotropic and ornamental purposes.
3	*Abrus precatorius*	Rosary Pea, Jequirity, Crab's Eye, Precatory	Seeds, Leaves.	Throughout central India.	Abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, febrifuge, laxative, purgative, refrigerant, and sedative	Unani tradition	Seeds disturb the uterine functions and prevent conception in women. The oil extracted from seeds is said to promote the growth of human hair.
4	*Cinnamomum zeylanicum*	True Cinnamon, Laurus	Bark, Leaf, Leaves	It is widely cultivated in Srilanka & India.	Indian and Sri Lankan cooking Cinnamon is used as a common spice, not only for sweets but also as an integral part of the spice mixture known as 'curry powder'.	Ayurveda Siddha	Cinnamon stimulates the urinary tract and can be used for problems of the kidneys, edema, and urinary retention
5	*Oxalis corniculata*	Yellow Wood Sorrel, Indian Sorrel	Whole Plant	Common in damp shady places, roadsides, plantations, and lawns in India.	Anthelmintic , anti-inflammatory, astringent, depurative, diuretic, emmenagogue, febrifuge, lithontripic,	Unani Ayurveda	It is used in the treatment of influenza, fever, urinary tract infections, enteritis, diarrhea, traumatic injuries, sprains, and poisonous snake bites.
6	*Rosa indica L.*	Red Rose, Pink Rose	Red Rose Petals, Pink Rose Petals,	cultivated throughout India	Astringent and vulnerary and useful in intestinal ulcers, rickets, hemorrhages and diarrhoea	Unani Tradition	The leaves are useful in treating wounds, ophthalmic, hepatopathy, and hemorrhoids.
7	*Holarrhena antidysenterica L.*	Bitter Oleander, Tellicherry Bark	Bark, Seeds	Grows wild in mountains	Astringent herbs		It is one of the best drugs for diarrhea. In chronic diarrhea & to check blood coming from the stool. It is a well-known herb for amoebic

							dysentery and other gastric disorders.
8	*Lantana camara*	Lantana Weed, Wild Sage, Shrub Verbena, Yellow Sage.	Leaves, Lantana Fruits.	Cultivated in specific areas.	Antirheumatic herbs	Ayurveda Siddha	It has several uses, mainly as herbal medicine and in some areas as firewood and mulch. The leaves are used to relieve itching. Other uses are against flu, colds, coughs, fevers, yellow fever, dysentery, and jaundice.
9	*Piper longum L.*	Long Pepper, Pipli	Fruit, Root, Stem	Most deciduous to evergreen forests	Antibacterial herbs	Ayurveda	Good for aromatic, stimulant, carminative,constipation, gonorrhea, paralysis of the tongue, advised in diarrhea, cholera, scarlatina , chronic malaria, viral hepatitis
10	*Thymus vulgaris*	Thyme, Garden Thyme	Whole Plant	Indigenous to Mediterranean regions and southern Europe, but prospers almost anywhere in a temperate climate.	Anthelmintic, strongly antiseptic, antispasmodic, carminative, deodorant, diaphoretic, disinfectant, expectorant, sedative, and tonic.	Ayurveda Siddha	Used in the treatment of dry coughs, whooping cough, bronchitis, bronchial catarrh, asthma, and treatment of tonsillitis, gum diseases, rheumatism, arthritis, and fungal infections.
11	*Zingiber officinale rosc.*	Calamus, Sweet Ginger, Ginger Root, Sonth (dried)	Fresh And Dried Rhizomes.	Southeast Asia and throughout India.	Antioxidants, antibacterial, anti-inflammatory, and carminative.	Ayurveda Siddha Unani	Proved as prophylactic of nausea and vomiting associated with motion, sickness, seasickness, and pregnancy. Known for its gastrointestinal benefits and as an anti-inflammatory and carminative.

Table.4 Weightage Matrix Scores for herbal plants screened based on binary matrix scores (Scores ≥ 3)

Name of Plant / BAP	ABB OF PLANT	BAP1	BAP2	BAP3	BAP4	BAP5	Weightage Score
		PI	**T.P**	**HB.P**	**IgA1**	**P.L**	
Artocarpus heterophyllus	AH	+	+	+	+	+	17.9
Cinnamomum zeylanicum	CZ	+	+	+	+	+	17.9
Oxalis corniculata	OC	+	+	+	+	+	17.9
Rosa indica L.	RP	+	+	+	+	+	17.9
Argyreia nervosa	AN	+	+	+	+	+	17.9
Abrus precatorius	AP	+	+	+	+	+	17.9
Holarrhena antidysenterica L.	HA	+	+	+	+	+	17.9
Lantana camara	LC	+	+	+	+	+	17.9
Piper longum L.	PL	+	+	+	+	+	17.9
Thymus vulgaris	TV	+	+	+	+	+	17.9
Zingiber officinale rosc.	ZO	+	+	+	+	+	17.9

Table 5 Fuzzy Set Membership Analysis for herbal plants screened based on Weightage Matrix scores

S.NO.	HERBAL PLANT	μS	OPTIMIZED SCORE
1	*Actocarpus heterophyllus*	1	+++++(5)
2	*Cinnamomum zeylanicum*	1	+++++(5)
3	*Oxalis corniculata*	1	+++++(5)
4	*Rosa indica L.*	1	+++++(5)
5	*Argyreia nervosa*	1	+++++(5)
6	*Abrus precatorius*	1	+++++(5)
7	*Holarrhena antidysenterica L.*	1	+++++(5)
8	*Lantana camara*	1	+++++(5)
9	*Piper longum L.*	1	+++++(5)
10	*Thymus vulgaris*	1	+++++(5)
11	*Zingiber officinale rosc.*	1	+++++(5)

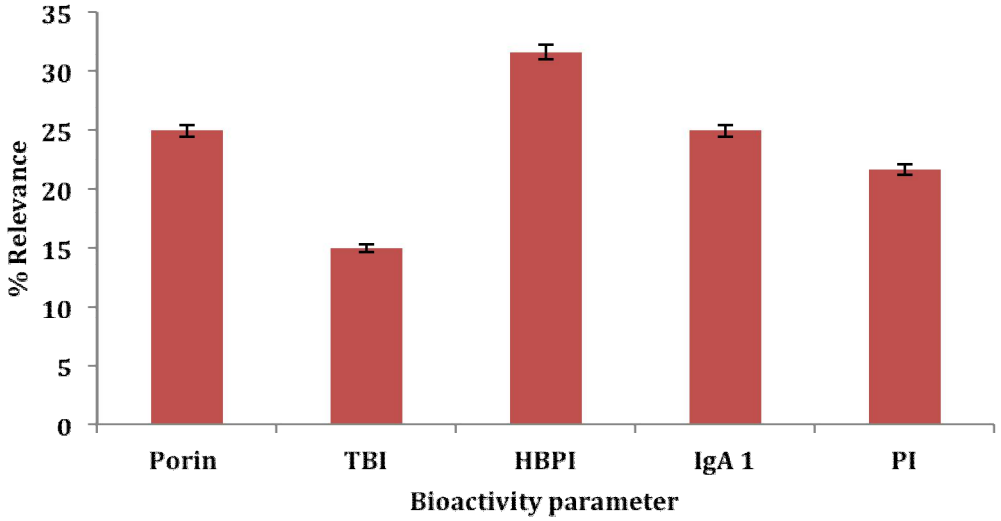

Figure1: Percentage relevance of identified bioactivity parameters

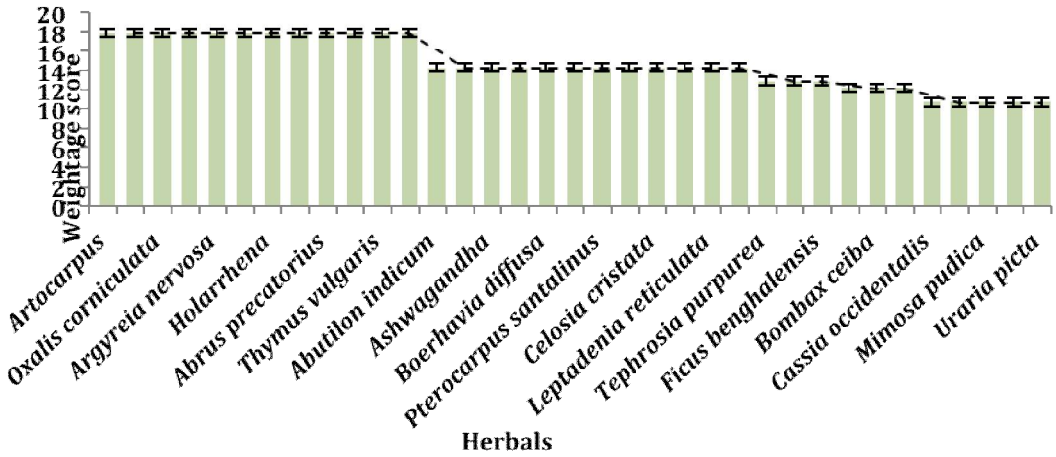

Figure 2: *Fuzzy Set optimization for herbals*

DISCUSSION

Gonorrhoeae is a sexually transmitted disease caused by the diplococci bacteria *Neisseria gonorrhoeae.* This disease gives rise to complications such as skin pustules, septic arthritis, meningitis, infertility and inflammation of reproductive organs, and prostate cancer in men (11). This disease may spread to adjacent organs like liver tissues and may also be passed on from mother to foetus, which causes blindness or conjunctivitis to the foetus, and WHO estimates that 88 million Gonorrhoeae cases occur every year. Centre for Disease Control and Prevention recommends dual drug therapy for the treatment of this prevalent disease (12). Various drugs used to treat are Ceftriaxone, Azithromycin, Cefixime,

Doxycycline and Erythromycin ophthalmic (for neonates). However, these drugs may only terminate the infection, without repairing the permanent damage done by the bacterial disease.

Due to these limitations of synthetic drugs, natural drugs made from natural plant products are gaining popularity as an alternative and effective approach for the treatment (13,14). The present study shows an *in-silico* model which is a rationale-based method for the selection of a plant that can aid the research for inhibiting and treating this communicable bacterial disease. PubMed was used as a random search engine. The bioactivity parameters were also selected concerning their role in this disease (15, 16)

The binary matrix approach was used in this study to identify a plant based on the all or none principle and hence plants were scrutinized based on their scores. Those whose median cut-off value was 12.5 were selected as herbs having immense potential for a therapeutic agent. This was followed by weightage matrix and fuzzy score set analysis which further identified 11 plants that had μS = 1 include *Actocarpus heterophyllus, Cinnamomum zeylanicum, Oxalis corniculata, Rosa indica L., Argyreia nervosa, Abrus precatorius, Holarrhena antidysenterica L., Lantana camara, Piper longum L., Thymus vulgaris, and Zingiber officinale rosc*. These identified herbal compounds might be able to manage this disease, possibly control it and the complications accompanied with it. These selected herbs can be further studied at *the in-vitro* and *in-vivo level*s to fully establish as a therapeutic agent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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