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Antibacterial activity of the terrestrial fern *Lygodium flexuosum* (L.) Sw. against multidrug resistant enteric- and uro-pathogenic bacteria

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ABSTRACT

Objective: To investigate antibacterial properties of the terrestrial fern *Lygodium flexuosum* (*L. flexuosum*) obtained from Kalahandi district, Odisha against enteric- and uro-pathogenic bacteria isolated from clinical samples. **Method:** Frond-extracts of *L. flexuosum* were obtained by the cold percolation method using four solvents, petroleum ether, chloroform, methanol and water. Antibacterial potencies of concentrated cold frond-extracts were tested by the agar-well diffusion method against 7 multidrug resistant (MDR) bacteria of which, 2 were Gram-positives, methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus faecalis* (VRE), and 5 Gram-negatives, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. **Result:** The cold-water frond-extract had the best antimicrobial activity against 7 MDR bacterial isolates, compared to extracts with other solvents. Values of zones of inhibition against MRSA and *P. mirabilis* were the highest, 29 mm. Zones of inhibition against VRE and *P. aeruginosa* were 25 mm, while those were 23 mm against *E. aerogenes* and *E. coli*. The least size of zone of inhibition 19 mm was recorded against *K. pneumoniae*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of active frond-extracts with water, chloroform, methanol, and petroleum ether were recorded. For the water extract, the MIC value 1.562 mg/mL against MRSA and *P. mirabilis*, but the value 3.25 mg/mL against VRE, *E. aerogenes* and *P. aeruginosa*, while the value of 12.5 mg/mL against *K. pneumoniae* were recorded. MBC values were the least with chloroform-extracts, with the range 12.5 for 6 bacteria, excluding *P. aeruginosa* for which, the value 25 mg/mL was recorded as MBC. **Conclusions:** Phytochemical analysis of the water-extract of *L. flexuosum* confirmed the presence of glycosides and carbohydrates, but alkaloids, terpenoids, steroids, saponins, tannins, and flavonoids were absent. *L. flexuosum*, being a fern, is a suitable non-microbial source of antimicrobial for MDR strains of major enteric and uro-pathogens.

1. Introduction

Pteridophytes (vascular cryptogams or ferns) enjoy a ubiquitous distribution in India, but they are generally shade-loving plants. The fern *Adiantum* is used in Indian Ayurveda and Unani systems and the fern *Lycopodium* is used in homeopathic system. Ethnobotanical accounts of 20 ferns have been documented from Kumaun Himalayas[1], and medicinal properties of 16 fern species of Western Ghats, India also have been recorded[2]. Similarly, ethnobotanical accounts of 13 ferns including the *Lygodium flexuosum* (*L. flexuosum*) (L.) Sw. (Family, Schizaeaceae; common name, maiden hair creeper, a rhizomatous perennial terrestrial fern, common in Southeast Asia and Australia), have been recorded as increasing memory power[3]. Traditionally, the whole plant of *L. flexuosum* is used for hepato-fibrosys, cough, rheumatism, sprains, scabies, eczema, jaundice including wounds and skin diseases; fresh roots and fronds

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are used to treat boils and the plant is as anti-inflammatory, too[4]. It is the principal weed of Malayasia.

A report on antibacterial activity of methanolic leaf-extract of *L. flexuosum* had records of *in vitro* control against drug-sensitive strains of Gram-positives, *Micrococcus luteus* and *Staphylococcus aureus*, and Gram-negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli* - all from Microbial Type Culture Collection or MTCC strains; frond- and petiole-extracts of the fern obtained with petroleum ether, acetone and water had no antibacterial activity on these bacteria, but rhizome-extracts with these three solvents had significant antibacterial properties[5]. Moreover, ferns, *Adiantumcapillus veneris*, *Adiantum ncisum*, *Adiantum lunulatum*, *Actiniopteris radiata*, *Araiostegia pseudocystopteris*, *Athyrium pectinatum*, *Chelienthes albomarginata*, *Cyclosorus dentatus*, *Dryopteris cochleata*, *Hypodematium crenatum*, *Marsilea minuta* and *Tectaria coadunate*, collected from Aravalli hills, Rajasthan, India had antibacterial activity against the phytopathogen, *Agrobacterium tumefaciens*, and human pathogens, *Salmonella arizonae*, *E. coli* and *Salmonella typhi* (all MTCC strains)[6].

There is a litany of well-known plants lending inimitable phytocompounds as processed and established medicines and quintessential drugs would be quinine from *Cinchona officinalis*, morphine from *Papaver somniferum*, reserpine from *Rauvolfia serpentina* and many more for several cataclysmic human ailments. Nevertheless, due to the spontaneous degradation of certain phytocompounds on storage, those are often ignored; the plethora of phytocompounds could address the drug-targeting crusade for infectious diseases due to intractable multidrug resistant (MDR) bacteria. Particularly, conflations of phytochemicals, as in crude plant extracts have been proving effectively in the control of MDR pathogens, in vitro, as repeatedly reported[7-9]. Now, the concept of use of phytodrugs is widely held by WHO[10], and its future is becoming deeply held, because of avalanche of pugnacious MDR pathogens, as a new paradigm of infection biology. Particularly, the situation of infection scenario has gone from abysmal to bad, for a few saturnine pandrug resistant pathogens (with strains resistant to all drugs of major classes of antibiotics of present day) [11]. For example, *S. aureus*, *P. aeruginosa* and *Acinetobacter baumannii* are noteworthy, since their resistant strains are circulated in communities and subtly flurry in hospitals causing clinical consternations[12]. Previously known as a harmless commensal, *S. aureus* is marked today as the ferocious superbug, MRSA among the torrent of pathogens, and MDR *P. aeruginosa* as well as MDR *A. baumannii* are labeled as the most notorious pathogens of urinary tract, causing frenzy morbidity and mortality, everywhere-from slums of developing countries to developed countries[8,13,14]. Sometimes, they precipitate exasperating episodes in public health[13,14]. Thus, when used with ingenuity, coalesced phytocompounds of a plant in crude extracts, which are covertly put into practice down the aborigine generations and the clandestine information recorded in ethnobotanical literature, would be opening prurient opportunities in the crusade against MDR pathogens.

L. flexuosum is a weed. Any plant with a characteristic set of unpalatable/aromatic phytochemicals cause aversion to grazing animals, eventually cause a plant to come up as a weed. Many ferns including *L. flexuosum* have unpalatable/aromatic phytochemicals and this plant particularly being rhizomatous grows perennially and succeeds as a weed. Intuitively stating, a successful weed would have phytochemicals suitable for the control of pathogens; thus, such plants need a microbiological evaluation, possibly with MDR bacteria. Ultimately, pure compounds could be improved for finesse by apothecary, as done for quinine for example.

The present study records antibacterial activity of frond-extracts of *L. flexuosum*, extracted with petroleum ether, chloroform, methanol and water, against 2 Gram-positive bacteria, methicillin resistant *S. aureus* (MRSA), vancomycin resistant *Enterococcus faecalis* or VRE, and 5 Gram-negatives, *Enterobacter aerogenes* (oxidase negative, catalase positive, indole negative and rod shaped)[7], *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* - all isolated from clinical samples. Further, *E. aerogenes* was reported causing sepsis at surgical sites, as well as its non-medical disturbances in food spoilage were recorded[15]; MRSA too is a supurative pathogen of surgical sites. Moreover, these Gram-negatives are nosocomially spreading pathogenic bacteria, causing opportunistic infections of gastrointestinal and urinary tracts through several spread-routes[16].

This paper describes antibiotic susceptibility of seven clinically isolated pathogens against 4 aminoglycosides, 2 β-lactams, 2 cephalosporins, 5 fluoroquinolones, 1 glycopeptide, 1 sulfonamide and 3 stand-alone antibiotics. In this study, 8 solvents (non-polar to polar) were used for search of bioactive frond-extracts, but with four solvents, petroleum ether, chloroform, methanol and water only active frond-extracts were obtained. These extracts were used for antibacterial properties and their preliminary phytochemical analyses too were done. This paper clearly elucidates the scientific basis of the traditional ethnomedicinal information of the plant as a source of 'non-microbial antimicrobial' with seven MDR pathogenic bacteria, elucidated never before with extracts of any fern.

2. Materials and methods

2.1. Collection of plants sample and preparations of plant extracts

Fronds of *L. flexuosum* (Figure 1) were collected from forest pockets of Kalahandi. Collected fronds were dried

and powdered and the powder-mass was stored in airtight polythene packs until use. For the cold extraction, four lots of 10 g of frond powders were dissolved in 100 mL volumes of four organic solvents, petroleum ether, chloroform, methanol and water in Tarson screw-cap bottles and were stored at 4 ℃ for 5 d. Each solvent-extract after centrifugation was dried using a rotary evaporator until a semisolid mass was obtained. Each extract was further stored in a small vial using 10% dimethyl sulfoxide (DMSO) at 4 ℃ until use.

Figure 1. *L. flexuosum*.

2.2. Isolation and identification of the bacteria

For obtaining bacteria, clinical samples, urine and stool were collected from in-house patients of this hospital. Samples were cultured in suitable media and the bacterial isolates were identified by using standard biochemical procedure, described previously for Gram-negatives[8] and Gram-positives[9]. Two Gram-positives (*S. aureus* and *E. faecalis*) and five Gram-negative bacteria (*E. aerogenes* Figure 2, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*) were isolated and were used in the study. Standards stains obtained from MTCC were used as reference controls for identifying clinical isolates[14].

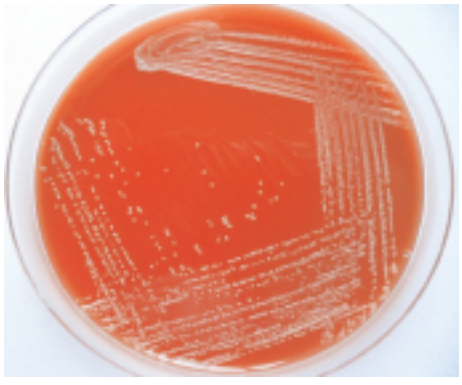

Figure 2. Colonies of *Enterobacter aerogenes* on blood agar.

2.3. Antibiotic susceptibility test

All isolated bacterial strains were subjected to antibiotic sensitivity test by Kirby-Bauer's/disc-diffusion method, described previously[16]. Eighteen antibiotics were used against Gram-positive bacteria, while 16 antibiotics were used against Gram-negatives (Figure 3).

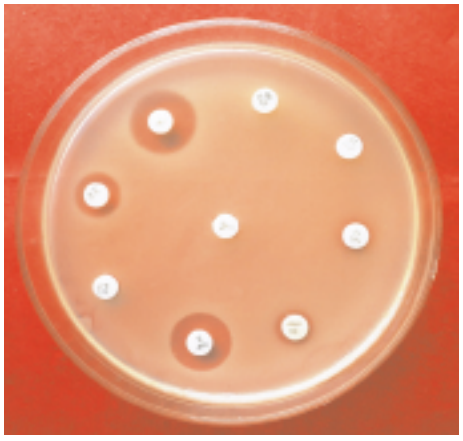

Figure 3. Antibiotic sensitivity of *E. coli* by disk diffusion method. Antibiotics (μg/disc): AK: amikacin 30; AMP: ampicillin 10; C: chloramphenicol 30; CIP: ciprofloxacin 5; C-OT: co-trimoxazole 25; CTX; ceftriaxone 30; NX: norfloxacin 10; Of: ofloxacin 5; PIT: piperacillin/tazobatcam 100/10.

2.4. Detection of MRSA, VRE and ESBL strains

The isolated strains of *S. aureus* and *E. faecalis* were subjected for 'chromogenic agar media test' and 'vancomycin screen agar plate test' for confirming their MRSA and VRE status, respectively, method as described previously[8]. Similarly, double disc diffusion synergy test was used for the determination of extended spectrum beta-lactamase (ESBL) producers in 5 Gram-negative bacteria[16].

2.5. Antibacterial activity test by agar-well diffusion method and determination of MIC and MBC

Antibacterial activity of four solvent extracts of fronds was monitored by the agar-well diffusion method, as described previously[8,9]. Linezolid 30 μg/mL and imipenem 10 μg/mL were used as controls, for Gram-positive and Gram-negative bacterial work, respectively, and 10% DMSO solution was the negative control. Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values of the active solvent extracts were determined, as described previously[17].

2.6. Phytochemical Screening

Preliminary qualitative phytochemical analyses of active extracts were done, to confirm the presence of phytochemicals, carbohydrates, saponins, flavonoids, steroids, terpenoids, tannins, alkaloids and glycosides, as previously described[17].

3. Results

A clinical isolate of *S. aureus* was found resistant to 14 of 18 antibiotics used. It was sensitive to antibiotics, ciprofloxacin and chloramphenicol, whereas intermediate or moderate sensitivity to antibiotics, vancomycin and tetracycline were recorded. Likewise, the Gram-negative *E. aerogenes* (Figure 2) and *E. coli* (Figure 3) was resistant to 13 of 16 antibiotics. It was sensitive to ciprofloxacin and chloramphenicol and moderately sensitive to tetracycline. Further, antibiograms of the rest other five bacteria were recorded (Table 1).

The cold water frond-extract of *L. flexuosum* (Figure 1) had the best antibacterial activity against 7 MDR isolates, compared to extracts with other solvents. Values of zones of inhibition against MRSA and *P. mirabilis* were the highest, 29 mm. The zones of inhibition against VRE and *P. aeruginosa* were 25 mm, while those were 23 mm against *E. aerogenes* and *E. coli*. The least zone of inhibition of 19 mm was recorded against *K. pneumoniae*. Similarly, zones of inhibition of extracts with chloroform and methanol were recorded; and frond-extract with petroleum ether registered the lowest antibacterial activity (Table 2).

MIC and MBC values of active frond-extracts were recorded with water, chloroform, methanol and petroleum ether as solvents. For the water extract, the MIC value of 1.562 mg/mL was recorded against MRSA and *P. mirabilis*, 3.25 mg/mL against *E. coli*; 6.25 against VRE, *E. aerogenes* and *P. aeruginosa*, while 12.5 mg/mL against *K. pneumoniae* was recorded. Likewise, MBC values of the water extracts were determined. A MBC value of 12.5 mg/mL was recorded against MRSA and *P. aeruginosa*, 25 mg/mL against VRE, *E. aerogenes*, *E. coli* and *P. mirabilis*, and 50 mg/mL against *K. pneumoniae*. Similarly, MIC and MBC values of extracts with petroleum ether, chloroform and methanol were recorded (Table 3).

Phytochemical analysis of the water extract of *L. flexuosum* confirmed the presence of glycosides and carbohydrates, but alkaloids, terpenoids, steroids, saponins, tannins, and flavonoids were absent. Similarly, phytochemical analysis of the petroleum ether-extract confirmed the presence of carbohydrates, but alkaloids, glycosides, terpenoids, saponins, tannins flavonoids and steroids were absent. Further, phytochemical analysis of the chloroform extract confirmed the presence of carbohydrates, but alkaloids, glycosides, terpenoids, saponins, tannins flavonoids, and steroids were absent, methanol extract confirmed the presence of glycosides, terpenoids, carbohydrates, tannins, flavonoids and steroids, but alkaloids and saponins were absent (Table 4).

Table 1

Antibiogram of selected clinically isolated bacteria monitored by the disc-diffusion method.

Bacterium	Susceptibility to prescribed antibiotics																	
	Aminoglycosides				β -lactams		Cephalosporins		Fluoroquinolones					Glycopeptide	Sulfonamide	Standalones		
	Ac	Ge	Am	Ak	Ox	Pit	Ce	Cf	Ci	Gf	Na	No	Of	Va	Co-t	Ch	Nf	Te
MRSA	R	R	R	R	R	R	R	R	S	R	R	R	R	I	R	S	R	I
VRE	R	R	R	R	R	R	R	R	S	R	R	R	R	I	R	S	R	R
E. aerogenes	R	R	R	R	Nd	R	R	R	S	R	R	R	R	Nd	R	S	R	I
E. coli	R	R	R	R	Nd	R	R	R	I	R	R	R	R	Nd	R	S	R	R
K. pneumoniae	R	S	R	R	Nd	R	R	R	R	R	R	R	R	Nd	R	S	R	R
P. aeruginosa	R	I	R	R	Nd	R	R	R	S	R	R	R	R	Nd	R	S	R	R
P. mirabilis	R	R	R	R	Nd	R	R	R	R	R	R	I	R	Nd	R	I	R	S

MRSA, methicillin resistant *Staphylococcus aureus*; VRE, vancomycin resistant *Enterococcus faecalis*; R, Resistant; S, Sensitive; I, moderately sensitive; Nd, Not detected. Antibiotics (μg/disc); Ac, amikacin 30; Ak, amoxyclav 30; Am, ampicillin 10; Ce, ceftriaxone 30; Cf, cefpodoxime 10; Ch, chloramphenicol 30; Ci, ciprofloxacin 5; Co-t, co-trimoxazole 25; Ge, gentamicin 10; Gf, gatifloxacin 5; Na, nalidixic acid 30; Nf, nitrofurantoin 300; No, Nofloxacin 10; Of, ofloxacin 5; Ox, oxacillin 30; Pit, piperacillin/tazobactam 100/10; Te, tetracycline 30;Va, vancomycin 30.

Table 2

Antimicrobial assay by agar-well diffusion method of different cold solvent extracts of leaves of *L. flexuosum* and antibiotics as reference control against isolated multidrug resistant bacteria (zone of inhibition in mm).

Strain	PE	Chloroform	Methanol	Water	Lz/Imp (μg/mL)
MRSA	10	21	23	29	26
VRE	10	15	19	25	29
E. aerogenes	10	22	23	23	25
E. coli	11	18	14	23	21
K. pneumoniae	08	12	22	19	23
P. aeruginosa	09	23	19	25	29
P. mirabilis	10	19	16	29	24

PE: petroleum ether; Lz: linezolid 30; Imp: imipenem 10.

Table 3
MIC and MBC values of bioactive frond-extracts of *L. flexuosum* against isolated multidrug resistant bacteria (mg/mL).

Strain	Petroleum ether		Chloroform		Methanol		Water	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
MRSA	6.25	25	3.125	12.5	3.125	25.0	1.562	12.5
VRE	25.00	50	6.250	12.5	6.250	25.0	6.250	25.0
E. aerogenes	12.50	50	6.250	12.5	3.125	50.0	6.250	25.0
E. coli	12.50	50	3.125	12.5	12.500	25.0	3.250	25.0
K. pneumoniae	25.00	50	1.562	12.5	6.250	12.5	12.500	50.0
P. aeruginosa	25.00	50	6.250	25.0	12.500	25.0	6.250	12.5
P. mirabilis	6.25	25	6.250	12.5	6.250	50.0	1.562	25.0

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration.

Table 4
Preliminary phytochemical analysis of frond-extracts of *L. flexuosum* extracted with different solvents.

Solvents	Alkaloids	Glycosides	Terpenoids	Carbohydrates	Saponins	Tannins	Flavonoids	Steroids
Petroleum ether	−	−	−	+	−	−	−	−
Chloroform	−	−	−	+	−	−	−	−
Methanol	−	+	+	+	−	+	+	+
Water	−	+	−	+	−	−	−	−

'+': presence of the phytochemical; '−': absence of the phytochemical.

4. Discussion

MBC values of frond-extracts with chloroform were recorded as the least value 12.5 mg/mL with all pathogens used, except *P. aeruginosa* that was well controlled by the water extract, of course. Antibacterial effectivity of extracts was as follows, chloroform > water > methanol > petroleum ether, based on MBC values, but based on zone of inhibition effectivity was water > methanol > chloroform > petroleum ether. Moreover, this weed as an antimicrobial was implicit from the additional finding that of four solvents used for extraction, with three (chloroform, methanol and water), frond-extracts registered controlling potency equal to two reference antibiotics used, in this study. Further, acute and sub-acute levels of crude leaf-extracts, with water, ethanol and n-hexane, of *L. flexuosum* using Wistar rats had been monitored; those extracts had no toxicity at 5 g/kg and 1 g/kg levels, at acute and sub-acute levels, respectively[18]. Thus, this plant could safely be recommended for further work for the use as complementary and alternate medicine (CAM), in the control of MDR pathogens. Eight types of compounds were detected including alkaloids, glycosides, saponins, phenolic compounds and flavonoids, during a phytochemical investigation of frond-extracts of *L. flexuosum*[19]. The effectivity of methanolic extract, as seen, is probably linked to the presence of a majority of secondary metabolites. Further work is needed to mark its individual/ idiosyncratic/ active compounds albeit, the synergistic effect on the control of MDR pathogens is impeccably proved herein. Obviously, no microbe how much well-equipped be it may with an armamentarium of drug resistant genes procured from genetic exchange mechanisms and/or mutated by induction, according to continual neo-Darwinian evolutionary mechanisms could win over a bandwagon of phytocompounds of eukaryotic origin. Moreover, there is an increasing trend of love for natural chemicals over synthetic ones as medicines today, for which plant products often are preferred. Due to certain unproven non-target adverse, yet non-toxic effects of certain plants on host, mainstream medicine practitioners circumspect about suggesting phytodrugs. As rigorous host toxicity testing is done before promoting a synthetic chemical as a drug, phytodrugs need to be tested similarly. However, this fern has to wait a long for the suave as a marketed/institutional medicine, since basically it is a weed.

Antioxidant activity of *L. flexuosum* using the chemical, 2,2-diphenyl-1-picrylhydrazyl (DPPH), radical scavenging activity had been documented using frond-extracts using several solvents; only the methanolic extract had been recorded to have the maximum activity with the inhibitory concentration 50 (LC_{50}) value of 5 μg/mL. A considerable amount biochemical work with nuclear magnetic resonance spectroscopy of compounds of the plant-extract had been recorded during monitoring phenolic contents, as gallic acid equivalents[20,21]. These two works emphasize that this plant is potent enough as source of drugs from lower plants. Moreover, compounds, dryocrasol, tectoquinone, kaempferol, kaempferol-3-α-D-glucosides, α-sitosterol, stigmasterol, o-p-coumaryl-drycrassol are present in *L. flexuosum*[22]. These chemicals could be followed further. Additionally, phytoconstituents of other ferns, three species of *Adiantum* and three species of *Christella* had been recorded[23].

Antimicrobial activity of ferns, *Dryopteris filix-mas*, *Lygodium altum*, *Salvina molesta*, *Salvina cuculata* and

Helminthostachys zeylanica from eastern India had been studied against *E. coli*, *Bacillus subtilis*, *Vibrio cholerae* and *K. pneumoniae*[24]. Antibacterial activities of frond-extracts with solvents, petroleum ether, methanol, chloroform, benzene and water of 5 ferns (*Adiantum caudatum*, *Angiopteris evecta*, *Pteris confusa*, *Pteris argyraea* and *Lygodium microphyllum*) had been monitored against the MDR phytopathogen, *Xanthomonas campestris*. Antibiotic sensitivity of this phytopathogen was recorded and it was found resistant to amoxicillin 25 μg/disc, chloramphenicol 30 μg/disc and penicillin 5 μg/disc[25]. Antibacterial properties of frond-extracts using the solvent mixture, methanol: dichloromethane at 1:1 with 5 fern species, *L. flexuosum*, *Selaginella bryopteris*, *Adiantum philippense*, *Dryopteris eochleata* and *Tectaria coadunate* had been recorded against human pathogens *Neisseria gonorrhea*, *S. aureus* and *P. aeruginosa* (all American Type Culture Collections, ATCC strains), with MIC values, 160, 140, 90, 80 and 60 μg/mL, respectively[26]. Antimicrobial activity of crude extracts of the epiphytic fern, Arthromeris himalayensis had been recorded against *B. subtilis* and *E. coli* by the agar well diffusion method[27]. The rhizome-extracts with ethanol, acetone, methanol and water of the fern *Drynaria quercifolia* had no inhibitory activity, but rhizome-extracts with diethyl ether had significant antifungal activity[28].

Drug resistance in pathogenic bacteria has been a matter of clinical consternation as a bacterium develops resistance to antibiotic/drug on application, at a faster rate than expected, in presence of a particular drug in a mechanism, 'positive selection pressure'[29,30]. In addition, there are several natural mechanisms of DNA exchanges, such as, bacterial transformation and conjugation that facilitate camaraderie amongst pathogenic and non-pathogenic bacteria for exchange of drug resistant genes. For example, the multiple antibiotic resistance (mar) locus of *E. coli* had been detected in many pathogenic bacteria even in the phylogenetically distant *Mycobacterium smegmatis*[31]. Such a situation leads to drug resistance in a pathogen to which a particular drug had never been applied. For example, *V. cholerae* was resistance to ampicillin, amikacin and co-trimoxazole as reported in our earlier study, but the former two antibiotics were never applied against it[10]. This signifies that the gain of drug resistance character occurred through some of genetic exchange mechanisms that are so fast and versatile that in sewages even, a transient contact between two cells results in a DNA exchange via conjugation. Further, free DNA from a lysed bacterium could enter another bacterial cell qualifying the later with extra drug resistance; bacterial transformation in sewage water had since long been demonstrated[32]. The issue on mechanism of development of drug-resistant strains, in general and urinary tract infecting pathogens in particular, was well-treated earlier[13,14].

Obviously, remedies to overcome this problem would be to destroy the chance for emergence of drug resistance by the combination therapy. Secondly, antibiotics/drugs are employed at levels below the host-toxicity-causing-concentrations, eventually letting a higher chance factor for emergence of individual resistant mutants. The concept of 'mutant preventive concentration' has been taken up for certain pathogens, e.g., *Mycobacterium tuberculosis* - letting space obliviously for the development of drug resistance in a lower range of a drug-concentration and life threatening situation due to drugs at its upper range. This has been exemplified in tuberculosis chemotherapy[30]. In this perspective, the role of CAM could be sought after. Phytomedicines being age-tested by ethnic people, those could be dependable in this situation. Scientifically, a clinician would be desirous in treating a patient with a pure chemical as a medicine and crude plant-extracts are not preferred. When public health perils come into existence with enteric- and uro-pathogenic bacteria as studied here, pursuing to the scientific exactitude with pure phytochemicals, unequivocally, would be a time consuming process. This fern appears as a potent source of non-microbial antimicrobials for further work, if scaled for finesse/propriety with endeavour by apothecary. Many common and lesser-known weeds are still unbeknown potential source of medicines.

Conflict of interest

We declare that we have no conflict of interests.

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