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Surveillance of multidrug resistance of 6 uropathogens in a teaching hospital and *in vitro* control by 25 ethnomedicinal plants used by an aborigine of India

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ABSTRACT

Objective: To evaluate antimicrobial potencies of 25 plants with reports on ethnomedicinal uses for infectious ailments by the aborigine Kandha tribe of Kalahandi district, Odisha state, India for urinary tract infections. **Methods:** Over a period of 6 months, multidrug resistant (MDR) strains of 6 uropathogenic bacteria *Acinetobacter baumannii* (*A. baumannii*), *Citrobacter freundii* (*C. freundii*), *Klebsiella oxytoca* (*K. oxytoca*), *Proteus mirabilis* (*P. mirabilis*), *Proteus vulgaris* (*P. vulgaris*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were isolated from clinical samples in a teaching hospital; their antibiograms were ascertained. Concentrated aqueous and ethanolic extracts of leaves and barks of plants were used for monitoring their antimicrobial potencies, by the agar-well diffusion method. Phytochemical analyses of plant parts were done. **Results:** All isolated bacterial strains were resistant to 15 antibiotics of 6 groups including β-lactams. From a surveillance of bacterial isolates, it was evident that the distribution of MDR strains of each was more in hospital acquired isolates than the community acquired ones. Both aqueous and ethanolic extracts of plants, *Aegle marmelos* (*A. marmelos*), *Azadirachta indica* (*A. indica*) and *Withania somnifera* (*W. somnifera*) were highly effective against MDR isolates of all these pathogens. Several plants were moderately effective during *in vitro* control of the pathogens. Plants, *Anthocephalus cadamba* (*A. cadamba*), *Cleistanthus collinus* (*C. collinus*) and *Oroxylum indicum* (*O. indicum*) were totally ineffective in the control of isolated MDR uropathogen. *A. indica*, *T. arjuna* and *T. alata* contained the full range of phytochemicals (alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids), which could be attributed to the significant anti-uropathogenic activities. **Conclusion:** Plants, *A. indica*, *A. marmelos*, *Cassia fistula* (*C. fistula*), *T. arjuna*, *Salvadora persica* (*S. persica*), *W. somnifera* and *Vitex negundo* (*V. negundo*), particularly could be useful for an use as complementary/ supplementary medicines for MDR uropathogens.

1. Introduction

Medicinal plants are used for health care by all sections of people in India-the disadvantaged section uses plants as crude folk-medicines unwittingly for all diseases always, and the elite, well-heeled mass uses those as processed herbal medicines for a specific disease, such as jaundice or constipation, etc. And in tide of love for natural products in place of synthetics, the use of some concoctions of plant parts is in practice for the general health conditioning/boosting[1]. Moreover, a torrent of plants has been investigated pharmacologically and chemically, till date, for the opening up of new medicinal opportunities with active principles; a large number of phytochemicals lend themselves explicitly for the use as drugs, in modern medicinal system[2]. From a survey by 'United Nations Conference on Trade and Development', it had been recorded that about a 33% of total drugs produced by industrial nations are plant based and about a 60% of those are for infections[3].

Among 60 000 Indian flowering plants, about 3 000 plants, approximately, are identified as ethnomedicine or folk-medicine, and of them about 1 500 plants are used in Indian Ayurveda, Unani and Siddho systems[4]. This country has tropical/ subtropical rain and mangrove forests with many

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plants, whose lesser-known ethnomedicinal uses have not yet been recorded[5]. And the traditional knowledge on the use of plants recorded from aborigines is not yet been used with scientific exactitude; but those could provide information and substance coveted by the modern medicinal system. Thus, there remains a missing link between the ethnomedicinal knowledge and their scientific exploitation, at all the time. This study is an attempt to minimize the missing link between uropathogens and a cohort of lesser-known plants of a sub-tropical forest, used by a typical aborigine.

Specifically aborigines living in the Odishan forest, similar to those of other developing areas/countries, maintain the ethnobotanical knowledge on medicinal plants in a surreptitious way orally, down the generations. But, the fear of fallibility in the use of the clandestine knowledge in locating/identifying plants with associated modalities of their use has increased, due to the lack of attention of young adults, for the slow creeping of modernism into their capsule-like closed society; and those may vanish even. Frequent surveys are undertaken in Odisha state for the record of the traditional knowledge on ethnomedicine[6,7]. Distressingly, the regular summer forest-fire causes colossal losses of plants, each year[8]. Secondly, as if adding fuel to fire, in tide of nation development, forest is unsustainably exploited for a myriad of non-timber forest products, timber and 'land clearing for the contour farming'. Further, forest is the base for the deplorable services of ecotechnology. All these factors cumulatively cause the creation of forest-fallows, eventually a virtual thinning and shape-shifting of natural forests occur. All plants generally and herbaceous ones specifically, majority being ethnomedicinal, suffer a rapid or rather a blasting diminution of diversity-phytodiversity; a priory, that must be a factor for the lack of attention of young adults on ethnomedicine.

According to several WHO reports and records on surveillance of hospitals of both developing and developed nations, it is consensus that the emergence of multidrug resistant (MDR) strains of pathogenic bacteria has now become commonplace, creating an uproar of threat in public health, as the appalling MDR strains get circulated in hospitals, hurtle to community and migrate far and wide in a country, covertly; consequently, there are rising numbers of episodes due to MDR pathogenic bacteria[9]. Specifically, urinary tract infecting (UTI) bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Acinetobacter baumannii* (*A. baumannii*), *Proteus* sp. and *Escherichia coli*, etc. in females mainly, gastrointestinal tract infecting *E. coli*, *Klebsiella* sp. and *Vibrio cholerae* (*V. cholerae*), etc., and the suppurative *Staphylococcus aureus* (*S. aureus*) cause concerns in the health domain to precarious standards, due to multiple resistances to drugs. A life-threatening situation occurs when multiple infections are detected in a body; but, when the marauding pathogens are scornfully MDR, the situation would become so complex and demanding that extrication from the conundrum would be a staggering victory for the patient. As reported, a high mortality figure is associated with MDR uropathogens, particularly with an ineffective empiric therapy[10]. Shenanigans of the cesspool of MDR uropathogens described in clinical literature with *Acinetobacter* as an epitome, infecting critically ill, hospitalized patients, and subsequent epidemics have become an increasing cause of concern in public health[11]. Infection due to MDR *P. aeruginosa* is too of overriding importance in hospitals because of its high decimation figures up to 81%[12], for example. Our recent surveillance derided MDR *P. aeruginosa* for its subtle infection dynamics[13]. MDR strains of *Proteus mirabilis* and other members of family Enterobacteriaceae are the leading cause of many infectious diseases, mainly UTI and the eventual commotion in tertiary and intensive care units[12]. MDR *Proteus vulgaris* (*P. vulgaris*) strains isolated from a patient with an upper UTI in Japan was resistant to quinolones, tetracycline and chloramphenicol[14]. As reported from Germany, verotoxinogenic *Citrobacter freundii* (*C. freundii*) was the causative agent of haemolytic uraemic syndrome and gastroenteritis[15].

A cohort of MDR *Klebsiella oxytoca* (*K. oxytoca*) isolates demonstrating resistance to imipenem, meropenem, extended-spectrum cephalosporins and aztreonam were identified[16]. Thus, there is a trend of emergence of increasing number of strains resistant to several antimicrobial agents in members of Enterobacteriaceae, influencing the prognosis and treatment of hospitalized patients. Consequently, their control cannot be forsaken solely on any one group of antibiotics in antimicrobial stewardship, nor can a new antibiotic be ossified, a priory in the empiric therapy for MDR uropathogens, with their well-known comorbidities and a high level of casualty.

An obsessive quest for avant-garde drugs for MDR pathogens leads to the exploration of alternate drug-sources always; and phytochemicals, obviously, remain as the palpable source of non-microbial antimicrobials, for which drug resistance would be impossible, for the inherent gamut of chemicals in plant extracts. So it was worthwhile to collect knowledge about several plants in use for infectious ailments by an ethnic mass of Indian tropical forest and the concomitant scientific verification of the recorded information with a cohort of MDR uropathogens isolated from clinical samples. This paper embodies ethnobotanical information of 25 medicinal plants and antibiogram of 6 UTI causing bacteria isolated in a hospital, and examines the antibacterial potentialities of these plants with folklore history of control over infectious diseases. It is anticipated that the embodied data would benefit apothecary for finesse of drugs in use and open possibility of some new use of phytochemicals as complementary/supplementary drugs against MDR uropathogens.

2. Materials and methods

2.1. Survey work

Reported plants were collected from the aborigine Kandha tribe at hills of the Eastern range of mountains of India, in the district Kalahandi, Odisha state in February 2010. About 50 respondents of 10 hamlets were interviewed, done in a

forest patch with a questioner and personal interview using the snowball technique in survey and sampling[17]; and the recorded information was documented (Table 1).

2.2. Preparation of plant extracts

Collected mature leaves/barks of plants were crushed to powders. A lot of 5 g of powder of a sample was dissolved in an aliquot of 25 mL of double distilled water and was sterilized for 30 min, before incubation at 4 ℃ for 72 h, with intermittent stirring. These steps were repeated for each plant sample. Water extracts were used directly for monitoring antibacterial properties *in vitro*. For an ethanolic extract, a lot of 5 g of each powdered plant material was soaked in an aliquot of 25 mL 80% ethanol for 72 h with the usual hand-shakings, and was filtered. The alcoholic-filtrate was concentrated in a rotary evaporator at 40 ℃, till a sticky mass was obtained that was weighed and dissolved in 1 mL of 10% v/v dimethyl sulfoxide (DMSO). For each plant sample, these steps were repeated and both extracts were stored at 4 ℃ until further use.

2.3. Collection of bacterial strains

Details of collection of bacteria are presented in Table 2; a total of 383 isolates, i.e., 189 isolates from hospital acquired (HA) and 194 isolates were from community acquired (CA) samples were collected. Six common uropathogens (*A. baumannii, C. freundii, K. oxytoca, P. mirabilis, P. vulgaris* and *P. aeruginosa*) were isolated during a span of 6 months.

2.4. Biochemical identification of isolated bacterial strains

The following tests were done in succession for clinical isolates: i. Catalase test with a small lump of bacteria and a drop of 3% H_2O_2. ii. Oxidase test: It was done a lump of bacteria on a filter paper disc, with tetramethyl-p-phenylenediamino dihydrochloride and the dye, indophenol for a change of colour of the disc from white to purple/dark purple within 10 s. iii. Indole test: to an aliquot of 5 mL 48 h old grown culture, an aliquot of 0.5 mL of Kovac's reagent (p-dimethyl amino benzaldehyde, isoamyl alcohol and HCl) was added; a formation of a cherry red ring at the top of the mixture indicated the indole production from tryptophan. iv. Methyl red test (MR test): To an aliquot of 5 mL of 48 h old culture in MRVP broth, 5 drops of methyl red was added as indicator; a red solution indicated the formation of organic acids as end products. v. Voges-Proskauer test (VP test): To an aliquot of 5 mL of 48 h old culture in MRVP broth, 10 drops of VP I reagent (5% α-napthol dissolved in absolute alcohol) and 2-3 drops of VP II reagent (40% KOH solution) were added, and the mixture was allowed to stand for 15-20 min for an appearance of red color (production of a neutral product, acetoin from the fermentation of glucose), but a yellow colour, on the contrary, indicated the negative result. vi. Citrate test: On inoculation onto a slant of Simon's citrate agar and incubation for 48 h at 37 ℃ of the test culture, a change of colour of the agar from green to blue indicated that citrate was used as the sole source of carbon. vii. Urease test: The test organism was inoculated to a slant of Christensen's urea agar. The production of ammonia as an excretory product increases the pH that changes the colour of the medium from off-white to pink, the positive result. viii. Triple-sugar-iron (TSI) test: The test organism was inoculated onto triple-sugar-iron agar slant and then a stab was made up to the butt of the slant, and was incubated for 37 ℃ for 48 h to check acid and gas production during fermentation of sugars. ix. Nitrate reduction test: An aliquot of 5 mL of nitrate broth was inoculated with a drop of 24 h old broth test culture and was incubated for 48 h at 37 ℃. From the development of the red colour within 30 s of adding to the culture a few drops of equal volumes of the reagent A (α-napthol 5 g and 30% acetic acid 1 000 mL) and reagent B (sulphanilic acid 5 g and acetic acid 1 000 mL) together, the positive result of capability of nitrate reduction was inferred. Bacterial strains were ascertained to taxa with results of biochemical tests (Table 3)[18].

2.5. Antibiotic sensitivity test

All bacterial strains were subjected to antibiotic sensitivity tests by the disc diffusion/ Kirby-Bauer's method, using a 4 mm thick Mueller-Hinton agar (MHA) medium[19]. An aliquot of 0.1 mL of 0.5 McFarland equivalents, approximately from an exponentially growing culture was spread on to agar for the development of lawn of any strain of a bacterium. Further on lawn-agar of each bacterial isolate on a plate, 6 high potency discs of prescribed antibiotics (HiMedia, Mumbai) were placed at equal distances from one another. Fifteen antibiotics of 5 different groups (Table 4) were used for determining the antibiotic sensitivity patterns of the 6 isolated bacteria. Plates were incubated for 18 h at 37 ℃ in a BOD incubator (Remi CIM-12S) to examine the zone of inhibition that was measured[20].

2.6. Antibacterial activity test by agar-well diffusion method

One strain from each bacterial species showing resistance to a maximum number of antibiotics was further used for the monitoring antibacterial potentiality of plants extracts, by the agar-well diffusion method[21]. Wells in bacterial lawn were punched for 6 mm deep and each well was based by 50 μL molten MHA. Further, wells were filled with 100 μL aliquots of 30 mg/mL solvent-extract of a plant (which was diluted from the original stock of plant extract of individual organic solvent, by 10% DMSO to 30 mg plant extract/mL, and that of the aqueous plant-extract with water). Plates were incubated at 37 ℃ for 24 h. Antibacterial activities were evaluated by measuring the diameter of zones of inhibition. The extracts causing a zone of inhibition 20 mm or more were considered highly active and plants having a zone of inhibition less than 20 mm were considered moderately active. Levofloxacin 5 μg/mL with an average size of zone of inhibition of 20 mm and DMSO 10% with no antibacterial activity were taken as reference controls.

2.7. Preliminary phytochemical analyses

2.7.1. Test for reducing sugars

The presence of free reducing sugars was ascertained by Fehling's test.

2.7.2. Test for anthraquinones

A lot of 0.5 g of the extract was shaken with an aliquot of 10 mL of benzene, filtered and an aliquot 5 mL of 10% ammonia solution was added to the filtrate and the mixture was shaken; the presence of a pink, red or violet colour in the ammoniac (lower) phase indicated the presence of anthraquinones[22].

2.7.3. Test for saponins

A lot of 0.5 g of an extract was dissolved in an aliquot 10 mL of distilled water in a test-tube was shaken vigorously for 30 s and then allowed to stand for 45 min. The appearance of a frothing on warming indicated the presence of saponins[23].

2.7.4. Test for flavonoids

To a portion of the dissolved extract, a few drops of 10% ferric chloride solution were added. A green or blue colour indicated the presence of flavonoids[24].

2.7.5. Test for steroids/terpenes

A lot of 500 mg of the extract from the rotary evaporator was dissolved in an aliquot of 2 mL of acetic anhydride and cooled at 0 to 4 ℃, to which a few drops of 12 N sulphuric acid were carefully added. A colour change from violet to blue-green indicated the presence of a steroidal nucleus[24].

2.7.6. Test for tannins

A lot of 0.5 g of the extract was dissolved in 5 mL of water followed by a few drops of 10% ferric chloride. A blue-black, green, or blue-green precipitate would indicate the presence of tannins.

2.7.7. Test for alkaloids

A lot of 0.5 g of plant extract was stirred with an aliquot of 5 mL of 1% HCl on a steam bath and filtered; to an aliquot of 1 mL of the filtrate, a few drops of Mayer's reagent was added, and to another aliquot of 1 mL of the filtrate, a few drops of Dragendorff's reagent were added. Turbidity or precipitation in tubes due to either of these reagents indicated the presence of alkaloids in the extract.

2.7.8. Test for resins

To an aliquot of 10 mL of the extract, an aliquot of 10 mL of 1% copper acetate solution was added and shaken vigorously, and a separate green colour indicated the presence of resin.

2.7.9. Test for glycosides

An aliquot of 5 mL of each extract was mixed with an aliquot of 2 mL of glacial acetic acid (1.048 g/mL), one drop of 1% $FeCl_3$ solution, and mixed thoroughly to which, an aliquot of 1ml of 12 N H_2SO_4 was added. A brown ring at the interface indicated the presence of glycosides[24].

3. Results

A. baumannii (Figure 1) was identified basing on its colony characteristics on nutrient agar and MacConkey agar along with the results of 9 biochemical tests. Its colonies were colourless smooth, opaque, raised and pinpoint on nutrient agar, and were non-lactose fermenting (NLF) on MacConkey agar (Table 2). Further, it was found positive to catalase, citrate and urease tests and negative to oxidase, indole, methyl red and nitrate reduction tests (Table 3). Similarly, the rest 5 bacterial isolates were identified (Tables 2 & 3).

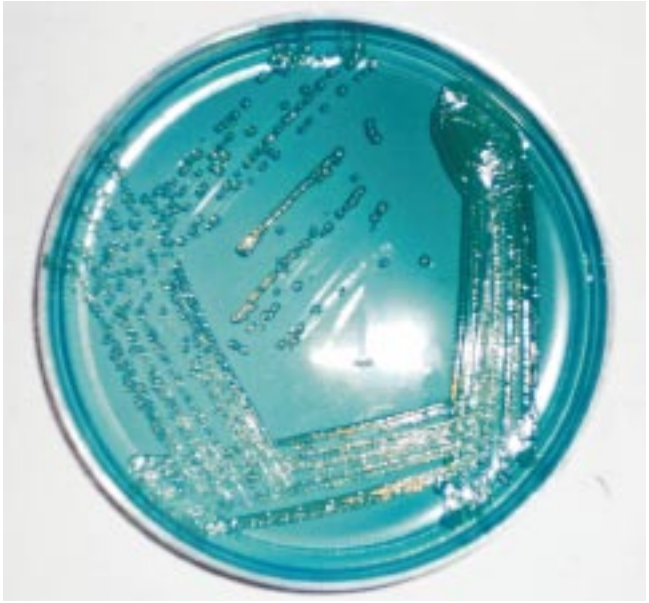

Figure 1. Non-lactose fermenting colonies of *A. baumannii* on CLED agar.

All bacterial strains isolated were found to be invariably MDR; one strain of each of 6 uropathogens was further selected for antibiotic profiling and for monitoring antibacterial activities of all cited plants. Several *P. aeruginosa* strains were found sensitive to amoxyclav, co-trimoxazole, levofloxacin and gatifloxacin whereas, those were found resistant to ampicillin, ceftriaxone, cefpodoxime, ciprofloxacin, gentamicin, imipenem, nalidixic acid, nitrofurantoin, norfloxacin, netillin, ofloxacin, piperacillin/tazobactam, at specified levels of each antibiotic. Similarly, for the antibiotic sensitivity patterns for other isolated UTI pathogens, separate strains of each were used and antibiogram was recorded (Table 4). It was discernible that *A. baumannii* was resistant to 14 out of 15 antibiotics used and *C. freundii* had resistance for 11 out of 15 antibiotics used.

A total of 38 (20%) and 60 (31%) isolates of *A. baumannii* were isolated from HA and CA samples, respectively; similarly, detailed numbers and percent values of rest 5 bacteria are presented (Table 5a). In HA clinical samples, *P. aeruginosa* isolates were 74 (35%), the highest value, whereas in CA, 60 (31%) clinical samples with *A. baumannii* were isolated; thus, it was the leading organism. Further, percent values of each of 6 pathogens resistant to individual drugs of 6 groups of antibiotics are also presented (Table 5b). For example, *P. aeruginosa* had the highest 92% resistance among HA isolated strains, while 89% resistance among CA isolates

Table 1.
Ethnomedicinal uses plants used.

Sl. No	Plant name	Family	Local name	Parts used	Ethnomedicinal uses
1	*Aegle marmelos* L. Corr.	Rutaceae	Bela	Leaf	It is used in constipation, dysentery and diarrhoea. Leaves are used for treating diabetes, jaundice, cholera, asthma and ophthalmic disorder.
2	*Anthocephalus cadamba* (Roxb.) Miq.	Rubiaceae	Kadamba	Leaf	Its bark is used of urinary infections and biliousness is used. It is used for diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds and ulcers.
3	*Argyreia speciosa* L.f.	Convolvulaceae	Brudha daraka	Leaf	Warm aqueous extract of *A. cadamba* leaves have been used to alleviate the wound healing and cuts.
4	*Azadirachta indica* L. Adelb	Meliaceae	Neem	Leaf	It used as vermifuge and antiseptic as it is antibacterial and antiviral in action (chicken pox). It is used in the treatment of acne.
5	*Bacopa monnieri* L. Pennell	Scrophulariaceae	Brahmhi	Leaf	It helps protect the stomach from ulcer formation. It is useful in diarrhoea and fevers, asthma and hoarseness.
6	*Butea monosperma* Lam. Taub	Fabaceae	Palasa	Leaf	It is useful diarrhoea, urine infections, leprosy, ulcers, tumours and skin diseases.
7	*Calotropis procera* (Aiton)W.T.Aiton	Asclepiadaceae	Arakha	Leaf	The powdered root controls asthma, bronchitis and antihelminthic. Its root-bark is used as a treatment for elephantiasis, leprosy, and in eczema. Leaves are useful intermittent fevers. Flowers are useful in asthma, catarrh, inflammations.
8	*Camellia sinensis* L Kuntze.	Theaceae	Chai	Leaf	It possesses antibacterial, antiseptic, asthma. It is helpful in skin disorders
9	*Cassia fistula* L.	Caesalpiniaceae	Sunari	Leaf	It is useful in skin diseases, burning sensations and syphilis. It is useful in boils, leprosy, and ringworm affection. It is useful in skin diseases, burning sensation, dry cough, bronchitis, dysentery and inflammations.
10	*Catharanthus roseus* L. G. Don	Apocyanaceae	Sadabihari	Leaf	It is used in case of nosebleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss cystitis, gastritis and enteritis, diarrhoea.
11	*Cissus quadrangularis* L.	Vitaceae	Hadajoda	Leaf	It is useful in eye and ear diseases and colic, leprosy, ulcers, tumors and skin diseases.
12	*Cleistanthus collinus* Hook.f. ex Planch.	Euphorbiaceae	Karla	Leaf	It is used as an antiseptic and against diarrhoea, amenorrhoea.
13	*Elephantopus scaber* L.	Asteraceae	Mayurachulia	Leaf	Roots and leaves are reported for diarrhoea, dysentery, swellings and stomach pain. Powdered with pepper it is applied for tooth-ache. Leaves are used in applications for eczema and ulcers.
14	*Ficus glomerata* Roxb	Moraceae	Dumer	Leaf	Leaves decoction are used against dysentery, diabetes, stomachache piles and diarrhoea.
15	*Glycyrrhiza glabra* L.	Fabaceae	Yasthi-madhu	Leaf	It is useful in cough, bronchitis, ulcer, fever, hoarseness of voice, skin diseases, eye diseases and pharyngitis. It is also applied on cuts and wounds
16	*Holarrhena antidysenterica* L Wall.	Apocyanaceae	Kutaja	Leaf/Bark	It is used for diarrhoea and skin diseases. The bark paste is mixed with cow urine and applies it in affected skin parts. In treatment of urinary troubles, the bark is given with cow milk. The bark is used in chest affections and it is a well known herb for amoebic dysentery.
17	*Moringa oleifera* Lam.	Moringaceae	Sajana	Leaf	It acts as potent antitubercular and used to cure liver and is useful in diarrhoea. It is also used in fever, inflammations, amenorrhoea, dysmenorrheal, cough, cold and eye diseases.
18	*Oroxylum indicum* L. Kurz	Bignoniaceae	Phaphen	Leaf, Bark	Scabies, leprosy, diarrhoea, pyorrhoea. During measles and swelling of body, a small piece of bark is rubbed in stone with water and applied all over the body and a spoon full is given orally to arrest further growth.
19	*Pterocarpus santalinus* Linn.f.	Fabaceae	Rakta-chandan	Leaf/Bark	It is used as an antiseptic, wound healing agent and anti-acne treatment. A decoction of fruit is used as an chronic dysentery.
20	*Salvadora persica* Wall	Salvadoraceae	Meswak	Bark	Leaves are useful in asthma, bronchitis, cough, painful tumors, verminosis. Shoots and leaves are used in treatment of cough and bronchitis. Tender twigs are used as toothbrush.
21	*Tectona grandis* L.f.	Lamiaceae	Teak	Bark	It is used as an antiseptic, wound healing agent and anti-acne treatment
22	*Terminalia alata* Heyne ex. Roth	Combretaceae	Sahaj	Leaf	For epilepsy, diarrhoea, dysentery aliquots of 20-30 ml of bark is given daily for a month or till symptoms disappear.
23	*Terminalia arjuna* (Roxb.) Wight & Arn	Combretaceae	Arjuna	Leaf/Bark	The leave extracts inhibits skin diseases and urinary infection. It is used as expectorant. It acts against skin aliments including acne.
24	*Withania somnifera* L. Dunal	Solanaceae	Ashwagandha	Leaf	It has been used in diseases such as rheumatism, leprosy and arthritis.
25	*Vitex negundo* L.	Verbrenaceae	Nirgundi	Leaf	The dried fruit is vermifuge and is also used in the treatment of colds, coughs, diarrhoea, dysentery and acne treatment.

Table 2.

Source of isolation and media used for isolation and maintenance uropathogenic bacteria from clinical samples and their colony characteristics.

Bacterium	Source	Media used	Colony characteristics
A. baumannii	Urine/HVS	Nutrient agar	Colourless smooth, opaque, raised and pinpoint
		MacConkey agar	Colourless smooth, opaque, raised, NLF
		CLED agar	Blue coloured opaque raised NLF
C. freundii	Urine	MacConkey agar	Late LF light pink after 48 h
K. oxytoca	Urine	MacConkey agar	LF, pink, mucoid
P. mirabilis	Urine	MacConkey agar	LLF light pink after 48 h
		Blood agar	Swarms on blood agar with β hemolysis
		CLED agar	Translucent blue
P. vulgaris	Urine	Blood agar	Swarms on blood agar with β hemolysis
		CLED agar	Translucent blue
P. aeruginosa	Urine/HVS	Nutrient agar	Large, irregular opaque with bluish green pigment

LF: Lactose fermenting; NLF: Non-lactose fermenting; LLF: Late lactose fermenting, HVS: Higher vaginal swab; CLED: Cysteine lactose electrolyte deficient.

Table 3.

Biochemical identification of the isolated uropathogenic bacteria.

Bacterium (MDR strains)	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate
A. baumannii	+	−	−	−	+	+	V	nd	−
C. freundii	+	−	−	+	−	+	−	A/A H_2S	+
K. oxytoca	+	−	+	−	+	+	+	A/A + Gas	+
P. mirabilis	+	+	−	−	+	+	+	K/A H_2S	+
P. vulgaris	+	+	−	−	−	+	+	K/A H_2S	+
P. aeruginosa	+	+	−	−	−	+	+	−	+

A/A H2S, Acid in slant and butt with hydrogen sulfide gas production; K/A H2S, Alkali in slant and acid in butt with hydrogen sulfide gas production; A/A Gas − Acid in slant and butt with gas production; nd, not done; V, variable; +, positive; −, negative.

Table 4.

Antibiotic susceptibility results of the selected clinically isolated urinary tract infecting organisms.

Bacterium	Susceptibility to prescribed antibiotics														
	Aminoglycosides		β-lactams				Cephalosporins		Fluoroquinolones					Sulfonamides	Synthetic
	Ge	Nt	Ak	Am	I	Pt	Ce	Cf	Ci	Gf	Le	Na	No	Cot	Nf
A .baumannii	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
C. freundii	S	R	R	S	I	I	S	S	R	R	S	R	R	R	S
K. oxytoca	R	R	S	R	R	R	R	I	S	R	S	I	R	R	R
P. mirabilis	R	S	I	R	R	S	R	R	R	R	S	R	R	R	R
P. vulgaris	I	I	R	R	R	R	R	R	R	R	S	R	R	R	R
P. aeruginosa	R	R	S	R	R	R	R	R	R	S	S	R	R	S	R

'R'- Resistant; 'S'- Sensitive; 'I'- moderately sensitive; Antibiotics (μg/disc): Ak: amoxyclav 30; Am: ampicillin 10; Ce: ceftriaxone 30; Cf: cefpodoxime 10; Ci: ciprofloxacin 5; Co-t: co-trimoxazole 25; Gf: gatifloxacin 5; Ge: gentamicin 10; I: imipenem 10; Le: levofloxacin 5; Na: nalidixic acid 30; Nf: nitrofurantoin 300; No: norfloxacin 10; Nt: netillin 30; Of: ofloxacin 5; Pt: piperacillin/tazobactam 100/10.

Table 5a.

Hospital acquired and community acquired accounts of uropathogens (total = 189+194=383) in a span of 6 months.

Bacterium	Number of isolates	
	Hospital acquired (*n*=189=100%)	Community acquired (*n*=194=100%)
A. baumannii	38 (20)	60 (31)
C. freundii	32 (17)	21 (11)
K. oxytoca	11 (06)	23 (12)
P. mirabilis	15 (08)	38 (19)
P. vulgaris	26 (14)	31 (11)
P. aeruginosa	74 (35)	31 (16)

Numbers in parenthesis are percentages of occurrence.

Table 5b.

Antibiotic resistance pattern of the isolated uropathogens in the span of six months.

Bacteria	Percent values of resistant isolates to individual antibiotics†															*t*-value
	Aminoglycosides			β-lactams			Cephalosporins		Fluoroquinolone					Others		
	Ge	Nt	Ak	Am	I	Pt	Ce	Cf	Ci	Gf	Le	Na	No	Co-t	Nf	
A. baumannii	39(21)	22(18)	45(23)	29(25)	18(14)	54(37)	24(17)	59(43)	65(41)	42(23)	26(21)	49(22)	43(27)	85(45)	72(56)	2.67
C. freundii	45(36)	35(12)	61(49)	49(35)	21(19)	31(25)	49(29)	69(37)	69(47)	55(25)	39(19)	42(35)	75(67)	61(23)	71(67)	2.46*
K. oxytoca	35(19)	18(14)	41(23)	27(17)	15(9)	61(26)	19(04)	47(23)	63(31)	38(32)	21(11)	47(34)	39(12)	81(64)	69(23)	2.91
P. mirabilis	27(14)	33(25)	38(31)	42(23)	11(–)	36(31)	15(11)	32(17)	61(29)	33(09)	18(11)	35(21)	34(26)	79(56)	64(45)	2.27*
P. vulgaris	17(14)	31(16)	45(41)	67(36)	15(–)	21(17)	53(43)	48(35)	32(12)	76(56)	46(34)	36(12)	31(13)	65(34)	55(37)	2.53
P. aeruginosa	81(65)	45(19)	66(59)	42(26)	27(–)	56(45)	47(23)	72(67)	77(61)	55(25)	69(56)	63(61)	56(47)	92(89)	85(76)	1.82*

Antibiotics (μg/disc): Ak: amoxyclav 30; Am: ampicillin 10; Ce: ceftriaxone 30; Cf: cefpodoxime 10; Ci: ciprofloxacin 5; Co-t: co-trimoxazole 25 (Sulfonamide class); Gf: gatifloxacin 5; Ge: gentamicin 10; I: imipenem 10; Le: levofloxacin 5; Na: nalidixic acid 30; Nf: nitrofurantoin 300 (Synthetic); No: norfloxacin 10; Nt: netillin 30; Of: ofloxacin 5; Pt: piperacillin/tazobactam 100/10. "†" numbers denote from HA isolates (n=189) and numbers in parenthesis "( )" denote from CA isolates (n=194). Tabulated Student's t-value are 1.701, at P<0.05, and 2.467 at P<0.01 levels. "*" denotes significance at P<0.01 level; degree of freedom=28.

Table 6a.

Antibacterial activities of selected medicinal plants by the agar well diffusion method.

Bacteria	Zone of inhibition by twenty five plant extracts (mm)												
	1*	2	3	4	5	6	7	8	9	10	11	12	13
A. baumannii	17(19)	_	12(15)	16(17)	_		19(23)	_	_	_	_	_	14(21)
C. freundii	13(21)	_	_	17(19)	_	16(19)	14(16)	18(21)	_	21(24)	_	_	_
K. oxytoca	18(21)	_	_	21(24)	_	_	19(21)	21(24)	19(22)	17(19)	15(17)	_	_
P. mirabilis	16(18)	_	18(21)	13(17)	17(21)	_	17(20)	19(22)	_	_	_	_	18(21)
P. vulgaris	12(16)	_	16(18)	16(18)	16(19)	_	16(17)	21(24)	_	_	_	_	16(18)
P. aeruginosa	15(20)	_	16(19)	20(23)	18(22)	21(23)	18(21)	13(15)	21(24)	_	11(14)	_	_

*The numbers in row represent name of plants listed in Table 1; **numbers in rows represent values of zone of inhibition due to aqueous extracts and numbers in parenthesis are values of zone of inhibition due to ethanolic extracts.

Table 6b.

Antibacterial activity of selected medicinal plants by the agar well diffusion method.

Bacteria	Zone of inhibition by twenty five plant extracts (mm)											
	*14	15	16	17	18	19	20	21	22	23	24	25
*A. baumannii***	_	22(24)	_	17(21)	_	_	_	_	_	_	16(19)	18(20)
C. freundii	16(19)	_	13(16)	18(19)	_	15(18)	22(24)	_	21(24)	_	18(21)	15(16)
K. oxytoca	_	_	15(17)	19(21)	_	_	15(17)	21(23)	15(17)	_	15(17)	_
P. mirabilis	_	18(21)	18(21)	15(21)	_	_	21(23)	_	_	16(18)	10(12)	17(21)
P. vulgaris	_	14(21)	16(18)	17(21)	_	_	16(19)	_	_	21(23)	21(22)	18(20)
P. aeruginosa	21(24)	_	19(21)	19(21)	_	21(25)	_	_	19(23)	18(21)	11(14)	16(19)

See foot note of Table 6a.

Table 7.

Detailed results of antibacterial activity of 25 plants obtained from agar well diffusion method.

Plant name	Parts used	Aqueous extract		Alcoholic extract	
		Highly effective	Moderately effective	Highly effective	Moderately effective
A. marmelos	Leaf	*A. baumannii*	*C. freundii*	*C. freundii*	*P. mirabilis*
		K. oxytoca	*K. oxytoca*		
		P. mirabilis	*P. aeruginosa*	*P. vulgaris*	*P. vulgaris*
		P. aeruginosa			
A. cadamba	Leaf	–	–	–	–
A. speciosa	Leaf	*P. mirabilis*	*A. baumannii*	*P. mirabilis*	*A. baumannii*
		P. vulgaris		*P. vulgaris*	
		P. aeruginosa		*P. aeruginosa*	
A. indica	Leaf	*P. aeruginosa*	*A. baumannii*	*P. aeruginosa*	*A. baumannii*
		K. oxytoca	*C. freundii*	*K. oxytoca*	*C. freundii*
			P. mirabilis		*P. mirabilis*
			P. vulgaris		*P. vulgaris*
B. monnieri	Leaf	–	*P. mirabilis*	*P. mirabilis*	–
			P. vulgaris	*P. vulgaris*	

			P. aeruginosa	*P. aeruginosa*	
B. monosperma	Leaf	–	*C. freundii*	*C. freundii*	–
C. procera	Leaf	*K. oxytoca*	*C. freundii*	*A. baumannii*	*C. freundii*
		P. aeruginosa	*P. vulgaris*	*P. mirabilis*	*P. vulgaris*
			P. mirabilis	*K. oxytoca*	
				P. aeruginosa	
C. sinensis	Leaf	*C. freundii*	*P. aeruginosa*	*C. freundii*	*P. aeruginosa*
		K. oxytoca		*K. oxytoca*	
		P. vulgaris		*P. vulgaris*	
		P. mirabilis		*P. mirabilis*	
C. fistula	Leaf	*K. oxytoca*	–	*K. oxytoca*	–
		P. aeruginosa		*P. aeruginosa*	
C. roseus	Leaf	*C. freundii*	*K. oxytoca*	*C. freundii*	*K. oxytoca*
C. quadrangularis	Leaf	–	*K. oxytoca*	–	*K. oxytoca*
			P. aeruginosa		*P. aeruginosa*
C. collinus	Leaf	–	–	–	–
E. scaber	Leaf		*A. baumannii*	*A. baumannii*	
			P. mirabilis	*P. mirabilis*	
			P. vulgaris	*P. vulgaris*	
F. glomerata	Leaf	*P. aeruginosa*	*C. freundii*	*P. aeruginosa*	*C. freundii*
G. glabra	Leaf	*A. baumannii*	*P. mirabilis*	*A. baumannii*	
			P. vulgaris	*P. mirabilis*	
				P. vulgaris	
H. antidysenterica	Leaf	–	*K. oxytoca*	*C. freundii*	*K. oxytoca*
			P. mirabilis	*P. mirabilis*	*P. vulgaris*
			P. vulgaris	*P. aeruginosa*	
			P. aeruginosa		
M. oleifera	Leaf	–	*A. baumannii*	*A. baumannii*	–
			C. freundii	*C. freundii*	
			K. oxytoca	*K. oxytoca*	
			P. mirabilis	*P. mirabilis*	
			P. vulgaris	*P. vulgaris*	
			P. aeruginosa	*P. aeruginosa*	
O. indicum	Leaf	–	–	–	–
P. santalinus	Leaf	*P. aeruginosa*	*C. freundii*	*P. aeruginosa*	*C. freundii*
S. persica	Bark	*C. freundii*	*K. oxytoca*	*C. freundii*	*K. oxytoca*
		P. mirabilis	*P. vulgaris*	*P. mirabilis*	*P. vulgaris*
T. grandis	Leaf	*K. oxytoca*	–	*K. oxytoca*	–
T. alata	Leaf	*C. freundii*	–	*C. freundii*	–
		P. aeruginosa		*P. aeruginosa*	
T. arjuna	Leaf	*C. freundii*	*K. oxytoca*	*C. freundii*	*K. oxytoca*
			P. aeruginosa	*P. aeruginosa*	
W. somnifera	Leaf	*P. vulgaris*	*P. aeruginosa*	*P. vulgaris*	*P. aeruginosa*
			P. mirabilis	*C. freundii*	*P. mirabilis*
			K. oxytoca		*K. oxytoca*
			A. baumannii		*A. baumannii*
			C. freundii		
V. negundo	Leaf	–	*A. baumannii*	*A. baumannii*	*C. freundii*
			C. freundii	*P. mirabilis*	*P. aeruginosa*
			P. mirabilis	*P. vulgaris*	
			P. aeruginosa		
			P. vulgaris		

to co-trimoxazole 25 μg/disc. Further, Student's *t*-test was applied to ascertain whether HA or CA samples had more MDR strains of each pathogen. There was statistically significant differences with each pathogen in HA and CA isolates at $P<0.05$. Additionally, for *C. freundii*, *P. mirabilis* and *P. aeruginosa* percent values had statistically significant differences, at $P<0.01$, even (Table 5b). It could be concluded that the distribution of MDR strains of each pathogen was more in HA isolates, invariably.

Table 8.
Preliminary phytochemical analyses of aqueous and ethanolic extracts of the plants.

Sl. No	Plants	Alkaloids	Glycosides	Terpenoids	Reducing sugars	Saponins	Tannins	Flavonoids	Steroids
1	*A. marmelos*	− (+)	+ (−)	+(+)	+ (+)	+ (−)	− (+)	+ (+)	+ (+)
2	*A. cadamba*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
3	*A. speciosa*	− (+)	− (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)	− (+)
4	*A. indica*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
5	*B. monnieri*	+ (+)	+ (+)	− (+)	− (+)	− (+)	+ (+)	+ (+)	−(+)
6	*B. monosperma*	+ (+)	+ (−)	+ (+)	− (+)	+ (−)	−(+)	+ (+)	+ (−)
7	*C. procera*	− (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)
8	*C. sinensis*	− (+)	− (−)	− (+)	+ (+)	− (−)	− (+)	− (−)	− (+)
9	*C. fistula*	+ (+)	− (+)	− (+)	+ (−)	+ (+)	+ (+)	+ (+)	− (−)
10	*C. roseus*	+ (+)	− (−)	+(+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (−)
11	*C. quadrangularis*	+ (+)	− (−)	− (+)	− (−)	− (+)	− (+)	− (+)	− (+)
12	*C. collinus*	− (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)	− (+)
13	*E. scaber*	− (+)	− (+)	− (+)	+ (+)	+ (+)	+(+)	+ (+)	− (+)
14	*F. glomerata*	+ (+)	− (+)	− (+)	+ (−)	+ (+)	+ (+)	+ (+)	− (−)
15	*G. glabra*	+ (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
16	*H. antidysenterica*	+ (+)	+ (+)	+ (+)	+ (+)	− (+)	− (−)	− (+)	+ (+)
17	*M. oleifera*	+ (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
18	*O. indicum*	− (+)	+ (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	−(+)
19	*P. santalinus*	− (−)	+ (+)	− (−)	+(+)	− (+)	+ (+)	+ (+)	+ (+)
20	*S. persica*	+ (+)	+ (+)	− (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)
21	*T. grandis*	− (−)	+ (+)	− (−)	+ (+)	− (+)	+ (+)	+(+)	+ (+)
23	*T. alata*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
23	*T. arjuna*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
24	*W. somnifera*	+ (+)	+ (+)	+(+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)
25	*V. negundo*	+ (+)	− (+)	+ (+)	+ (−)	− (+)	+ (+)	+ (+)	+ (+)

"+" sign denotes presence, and "−"sign denotes absence of the compound in a plant; signs outside denotes about a phyto-chemical in water extract, and sign in parenthesis ( ) denotes in ethanolic extract.

Figure 2. *Holarrhena antidysenterica.*

Figure 3. *Aegle marmelos.*

While monitoring the antibacterial properties of 25 plants, it was evident that both aqueous and ethanolic extracts of *Aegle marmelos* (Figure 3), *Azadirachta indica*, *Holarrhena antidysenterica* and *Terminalia arjuna* were highly effective against the all UTI causing bacterial isolates; plants, *Anthocephalus cadamba*, *Cleistanthus collinus* and *Oroxylum indicum* were totally ineffective to all strains (Tables 6a & 6b). The categorization of highly and moderately effective plant extracts are detailed (Table 7). In general, the ethanolic extracts had better/ significant antibacterial activity than the corresponding water extracts (Table 7).

Preliminary phytochemical analysis was done for both extracts of all the 25 plants. In plants, *A. indica*, *T. arjuna* and *T. alata* contained all the phytochemicals (alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids), which could be attributed to the recorded significant antibacterial activity. Certain extracts such as the water extract of *Bacopa monnieri* did not contain terpenoids, but its alcoholic extract contained terpenoids. Presence of such phytocompounds in individual extracts cumulatively redounds to the antibacterial activities of plants. The results of phytochemical analysis of all plants are recorded (Table 8).

4. Discussion

From the antibiograms of 6 uropathogens with 15 antibiotics, it could be concluded that these were floridly

MDR. Further, a litany of MDR strains, with multiple resistances to antibiotics in use belonging to all major classes, of the emblematic/major uropathogen, *A. baumannii* have been reported from Europe, North and South America and a few more tropical countries; so, a near pandrug-resistant (resistant to almost all commercially available antimicrobials, or PDR)[10] strains have emerged to notorious proportions.

MDR isolates of *C. freundii* were reported as carbapenem resistant[25], with a capability of the production of 'K. *pneumoniae* carbapenemase' (KPC) enzyme including the metallo-β-lactamase that are the common determinants of carbapenem resistance in Enterobacteriaceae, worldwide[26]. Both *P. mirabilis* and *P. vulgaris* are infamous for their diverse mode of spread, and MDR strains of both species are reported to be abundant at 84.6% and 93.4% among total isolates in a typical study from a developing country[27]. And from Israel, it was reported that MDR *P. mirabilis* was prevalent and carbapenem was the only available treatment option[28]. The antibiotic tigecycline was reported to be therapeutically active against MDR *K. pneumoniae* and MDR *A. baumannii* isolates, with an optimal dosing at MICs around 0.5 g/mL[29]. It had been reported that for the control of *P. aeruginosa*, a single antipseudomonad β-lactam antibiotic was as efficient as the combination treatment with a β-lactam and an aminoglycoside antibiotic[30]. In this study, three β-lactam antibiotics were used. It was found that 42% of HA isolates and 26% of CA isolates of *P. aeruginosa* were resistant to ampicillin. Similarly, 27% imipenem resistance in HA isolates and no resistance in CA isolates of *P. aeruginosa* were recorded.

As side effects, aminoglycosides cause the generation of free radicals in the inner ear causing a permanent damage to sensory cells and neurons[31]; in an animal model, toxicity of aminoglycosides had been shown to generate free radicals of oxygen and nitrogen, which trigger an apoptotic cascade[32]. Indeed, β-lactams cause minimal side effects, but drug resistance of the group has been found more with both Gram-positive and -negative bacteria than other groups; for the bacterial resistance to β-lactam antibiotics, three strategies are identified: the production of β-lactamase enzymes, the utilization of insensitive cell wall transpeptidase and the expulsion of β-lactam antibiotics from Gram-negative cell by efflux pumps. The new generation of 'extended spectrum β-lactamase' (ESBL) prevalent in a wide range of bacteria is an epitome of pathogen evolution to win the new generation of β-lactam derivatives.

Moreover, cephalosporins are too used for an effective control of uropathogens, but those cause skin reactions, urticaria, rash, exanthema, pruritus and Steven-Johnson syndrome, in a sizable fraction of patients[33]. Pragmatically, antimicrobials commonly used to treat uncomplicated UTI include the combination drugs: trimethoprim and sulfamethoxazole or trimethoprim and one of β-lactams/fluoroquinolones/nitrofurantoin/fosfomycin/tromethamine[34]. But reports on ineffective empiric therapy are amply available lending to the emergence of MDR uropathogens, for example from the US[35], hitching down at a spate of complicated comorbidities.

Initially, the UTI treatment is done with trimethoprim and sulfamethoxazole, but a treatment failure is followed by the treatment with fluoroquinolones. But in our study, maximum percentages of resistance to ciprofloxacin and nalidixic acid (both fluoroquinolones) were recorded. Succinctly, it could be stated that these 6 uropathogens with exaggerated values multiple drug resistance find their place in the bandwagon of nosocomial pathogens that overplay the infection dynamics covertly. Premature decimation in females by the grisly UTI is the eventual consternation in public health. Drugs from age-tested herbal remedies should not have side effects, as seen for antibiotics.

Naturally transferable *A. baumannii* had been identified, and a strain of *Acinetobacter* sp. with the plasmid, ADP1 had served as a model organism in genome analysis[36]. The natural competence of taking extracellular DNA in *A. baumannii* had been reported as a hundred times more than calcium chloride treated *E. coli*.

In fact, the natural competence of *Acinetobacter* strains was attributed to the presence of genes, com*FECB* and com*QLONM*[37]. The most famous gene cluster with 45 resistant genes, 'Abar1' resistance was recognized as an island of a size of 86Kb in the genome of MDR *A. baumannii*; further, it consists of a mobile genetic element, transposon and other genes that were previously identified in *Pseudomonas*, *Salmonella* and *E. coli*[38]. Thus, this versatile genome of *A. baumannii* has challenging disseminating mechanisms of DNA exchange with related and unrelated bacterial pathogens conferring the ability of emergence of MDR mutants. Distressingly, *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* cause the utmost comorbidities and frequent immature mortality, originally colonizing at the urinary tract; these could be cited etymologically as ferocious quintessential PDR uropathogens, to put in sotto voce[39].

Moreover, it has been shown in *E. coli* that the multiple antibiotic resistant (*mar*) mutants involve an activation of the regulatory *mar* locus that confers the drug resistance by altering the expression of multiple genes, leading to an alteration in the cell wall[40]. The *mar* locus was further identified in *Salmonella typhimurium* and *P. aeruginosa*[40]. The *mar* mutants were reported to be resistant usually to low levels of several antibiotics in current use. Within the clinical chemoprophylaxis, resistance to tetracycline and rifampicin had been first reported to have been mediated by the plasmid R222, which mutated and conferred easily resistance to fluoroquinolones[41].

The camaraderie of exchange of genetic elements between both related and phylogenically distant strains of pathogenic bacteria helps to acquire of multiple drug resistances. Eventually, one resistant cell when emerges at least, that as if a doppelgänger bourgeons in the presence any of the current antibiotics, and all sensitive cells of the marauding pathogen get replaced by its progeny all over the infected body. Consequently, the clinical management of MDR pathogens remains inherently slippery and fallible. Obviously, the aftermath of the emergence of a MDR strain, in due course, is its spread all over resulting at a high

decimation figure as reported for *P. aeruginosa*[11].

Of the 25 plants used herein, only *Ae. marmelos, B. monnieri, C. sinensis, C. quadrangularis* and *M. oleifera* do not have any report of toxicity on human body. Particularly *C. sinensis* and *M. oleifera* are edible plants. *M. oleifera* had been described in folk medicines for the treatment of tumor[42], which has given impetus to undertake work on UTI pathogens and in the present study this plant had promising results. All these non-toxic plants have been recorded to have *in vitro* controlling capacity on these 6 isolated MDR bacteria. Further, the iconic plant of India, *A. indica* has been reported to have the control over 33 strains of UTI organisms including *Proteus, Klebsiella, Enterobacter, Pseudomonas* and *Providencia*[43].

Salvadora persica has been the most common medicinal plant to maintain the oral hygiene in the global Muslim community and it has been reported to have *in vitro* control over *P. aeruginosa*, particularly the water extract[44]. *Holarrhena antidysenterica* had a good control over *P. aeruginosa*[45]. *W. somnifera* had a significant control over *P. aeruginosa* and *P. mirabilis*[46]. *Vitex negundo* has been reported to have *in vitro* control over *P. aeruginosa* and *P. vulgaris*[47]. *T. arjuna* had *in vitro* control over *P. aeruginosa* and *K. pneumoniae*[48]. Plants have many antimicrobial properties due secondary metabolites such as alkaloids, terpenoids, flavonoids and phenolic compounds, etc., and the practice of use of plants as complementary and alternative medicine is now on increase worldwide due to WHO directives depicting several preclinical and clinical studies that have provided the scientific basis of efficacy of many medicinal plants to treat infections[49].

In conclusion, the present study clearly elucidated that the 6 uropathogen isolated were resistant to 15 antibiotics used, giving a way to conundrum in management of public health, as UTI causes utmost comorbidities, at least during pregnancy and child birth. A scouring time for some avant-garde drug perhaps is the need of the time. So, supplementary/complementary remedy would be with phytochemicals, as exemplified from *A. indica, A. marmelos, T. arjuna, S. persica, C. fistula* and *V. negundo* to control each of the isolated MDR uropathogens *in vitro* in this study. When scaled up, pure chemicals of these plants would be of use in chemoprophylaxis for MDR uropathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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