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Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria

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ABSTRACT

Objective: To evaluate the antimicrobial potency of 20 non-edible and/or poisonous plants used by an aborigine tribe (Kandha) of Kalahandi district for infectious diseases. **Methods:** Over a period of 5 months from two hospitals, 10 pathogenic bacteria (*Staphylococcus aureus* (*S. aureus*), Acinetobacter sp., *Citrobacter freundii* (*C. freundii*), *Chromobacterium violeceum* (*C. violeceum*), *Escherichia coli* (*E. coli*), *Klebsiella sp.*, *Proteus sp.*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*) and *Vibrio cholerae* (*V. cholerae*) were isolated to pure axenic cultures from clinical samples. Water and ethanolic extracts of leaves and barks were concentrated before monitoring antimicrobial activity by agar-well diffusion method. **Results:** All bacterial strains isolated were multidrug resistant. Ethanolic extract of most plants had effective antimicrobial activity against all the isolated multidrug resistant bacteria. Plants, *Anthocephalus cadamba* (*A. cadamba*) and *Pterocarpus santalinus* (*P. santalinus*) had antibacterial effect on all used bacteria. Water extract of several plants too had effective antimicrobial activity for all bacteria used. Effective *in vitro* control of MDR strains of *Acinetobacter sp.*, *C. freundii*, *Proteus sp.* and *P. aeruginosa*, the most potential urinary tract infection causing organisms by plant extracts of all major plant used herein is recorded. MDR *C. violaceum* isolated from skin lesions was found to be resistant to imipenem, piperacillin-tazobactam and amoxyclav and was found sensitive to 13 plant extracts. **Conclusion:** Effective *in vitro* control of MDR strains of Acinetobacter sp., *C. freundii*, *Proteus sp.* and *P. aeruginosa*; enteropathogenic bacteria, *E. coli*, *S. typhi*, *Klebsiella sp.* and *V. cholerae* were found to be well controlled by all plant extracts used.

1. Introduction

The World Health Organization (WHO) estimated that about an 80% population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs[1]. Particularly in rural India, uses of raw plant products as well as some concoction of Ayurvedic medicines are sought after to a great proportion, because of cheap availability, and in urban areas too those are popular[2]. Despite the advent of modernism in medicinal system in the 21st century, poverty-stricken and marginalized aborigine-folks (tribals) of India, living in forest patches, particularly, are still practicing the art of the use of crude herbal products as medicines[3-6]. In tribal-India, the clandestine knowledge of 'medicinal plants and their uses' is transmitted down the generations, which sometimes, becomes a risky affair due to the advent of the modernism itself that affects the attention for knowledge on plants and their identifications by young adults in forest floor[3,6]. It has been estimated that in the Indian subcontinent, about 45 000 species of wild plants are present, of which about 7 500-8 000 species of medicinal plants are used in tribal healthcare needs, and only about 1 500 plants are in use in Indian Ayurveda, Unani and Siddho systems, largely for elite mass[2].

Furthermore, the integrity of the rich phytodiversity typical to a tropical forest is at stake in India for reasons signposted: 1. increasing pressures on forest due to iniquitous and unsustainable human invasion for a myriad of forest-products, mainly timber; 2. the unwitting and unavoidable regular cataclysmic episodes of forest-fire in summer on a colossal scale due to the fire-catching milieu itself;

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and 3. intentionally fire used for clearing land patches for more crop-lands (shifting cultivations)[7,8]. Not surprisingly, creation of forest-fallow is so common with eventual shape-shifting and maiming of vegetations. Thus, continual surveys to record the traditional medicinal knowledge used by aborigine people is to be undertaken often in an obsessive quest in this sub-continent, not least because formal and institutional inventory of phytodiversity of Indian Eastern Ghats of mountain ranges is not available, but probably for the fear of extinction of nearly unknown or lesser known forest-plants, for future planning of conservation strategies. It is easier said than done in any country.

Secondly, resistance of pathogenic bacteria to antibiotics is of high clinical concern. Rather the concept of the control of drug resistance is widely held today. A suitable epitome is the superbug, multidrug resistant (MDR) *Staphylococcus aureus* (*S. aureus*) in the human health domain worldwide, as its different strains or rather avatars have generated β-lactamase activities in degrading all sorts of penicillin derived antibiotics, in addition to resistance to other groups/generations of antibiotics[9]. Multidrug resistance of *Staphylococcus*, *Pseudomonas*, *Escherichia* and a few more pathogenic bacteria to a wide range of antibiotics has been reported to have been due to non-prudent uses of same antibiotics against infections of food- and pet-animals worldwide[10] and man. Several methicillin-resistant *S. aureus* (MRSA) strains carry resistance markers for other antibiotics and instances of resistance up to 23 antibiotics in some strains have been reported[11]. The emergence of vancomycin resistant strain (VRSA-vancomycin resistant *S. aureus*) is of further concern. Today, the management of the camaraderie of MDR strains of both Gram-negative and Gram-positive pathogens has become increasingly difficult because of the β-lactamase production in *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Neisseria*, *Salmonella*, *Haemophilus* and a few more pathogens[9], and pan-drug resistance to different classes of antibiotics in Gram-negative ones (12). Meek appreciation of failures in control of MDR strains would be inhuman, which generates the impetus on a systematic global search for new drugs from natural resources like plants, worldwide[12,14]; chemicals from plants could be chosen for control in a future crusade against MDR pathogens. Moreover, accumulated ethnomedicinal reports of different countries lend themselves well to the basic information needed for further work on drug-targeting against MDR pathogens[14].

This paper is anticipated to provide a scientific/microbiological verification of a cohort of twenty non-edible and/or poisonous plants reported from a typical Indian aborigine, the Kandha tribe of Kalahandi, as curing agents for different infectious diseases. As many as ten MDR pathogenic stains isolated from clinical samples of two teaching hospitals are used for monitoring antibacterial activities of 20 plants. The rationale of the study was to examine the reported medicinal plants against MDR bacteria that prove as notorious communal and nosocomial pathogens. The consortia of several interchanging lines of clonal nexuses yielded a gamut of MDR bacteria that are grimmish, ever-changing incarnations of pathogens developed insidiously, blitzkrieging patients of all age groups and bringing together comorbidities, eventually ending up in the terminal infecting illness in immunocompromised/aged patients. Thus, consternation in clinical managements is on the rise. Information on plants of a tropical forest embodied herein would help in the development of future antimicrobials against these appalling pathogens with a spectrum of artifices for burgeon due to naturally operative means of mutation and genetic recombination; is this the reason that ossification of any antibiotic/drug for any subtle pathogen is not possible.

2. Materials and methods

2.1. Survey work

Deciduous and scrub forest areas are too interspaced in the ever-green vegetations of Kalahandi district (19° 40′ N 83° 00′ E), which is one of the aborigine people inhabiting forest districts of Orissa state, in the Eastern Ghats of mountains of India. Plants reported (Table 1) were collected from Eastern Ghats between Junagarh and Bhawanipatna in the district Kalahandi, Orissa state in February 2010.

2.2. Preparation of plant extracts

Collected, dried plant samples (leaves and barks) in small pieces were crushed to powder form; a lot of 5 g of powders from a sample was dissolved in an aliquot of 25 mL of sterile distilled water and incubated at 4 ℃ for 48 h. The suspension was filtered and the filtrate was made up to a volume of 25 mL with sterile distilled water. Filtrates (water extracts) were used directly for studying their antibacterial properties. For 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 and 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) (Table 2) and those were stored at 4 ℃ until further use.

2.3. Bacterial work

Bacterial strains isolated and used were the Gram-positive, *S. aureus* and nine Gram-negatives, *Acinetobacter* sp., *Chromobacterium violeceum* (*C. violeceum*), *Citrobacter freundii* (*C. freundii*), *Escherichia coli* (*E. coli*), *Klebsiella* sp., *Proteus* sp., *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhi* (*S. typhi*) and *Vibrio cholerae* (*V. cholerae*). All these bacteria were directly collected from clinical

samples of Sum Hospital, Bhubaneswar and SCB Medical College, Cuttack, using an appropriate medium specific for a bacterium (Table 3), and were further isolated to pure (axenic) cultures before performing biochemical characterization of pathogenic strains[15]. Indian MTCC/NCTC (Microbial Type Culture Collection/National Collection of Type Cultures) standard drug sensitive strains of several bacteria were used in parallel: *S. aureus* NCTC 6571, *C. freundii* MTCC 1658, *E. coli* NCTC 10418, *Proteus* sp. MTCC 1771, *S. typhi* MTCC 733, *P. aeruginosa* NCTC 10662, *V. cholerae* MTCC 3905. Standard strains of *Acinetobacter* sp., *C. violeceum* and *Klebsiella* sp. were not available.

2.4. Biochemical tests

For pure-culture samples of Gram-positive cocci, catalase, oxidase and coagulase tests were performed. The catalase test was done with a drop of 3% H_2O_2 for affirmation. For the oxidase test, a sample of bacterial colony was rubbed onto a filter paper, impregnated with tetramethyl-p-phenylenediamine dihydrochloride and the dye indophenol. Change of colour of the filter paper to purple/dark purple within 10 s indicated oxidase activity. For the coagulase test, a lump of test organism was emulsified with a drop of normal saline water (0.89%). A drop of human blood serum was added to the suspension; clumping of cells was observed within 10 s, which confirmed the presence of bound coagulase enzyme. When a sample of Gram-positive cocci responded positively to both catalase and coagulase tests, it was confirmed to be *S. aureus*.

For pure-culture samples of Gram-negative bacilli, the following tests were done in succession. 1. Indole test: To an aliquot of 5 mL 48 h old grown culture (test culture), an aliquot of 0.5 mL of Kovac's reagent (p-Dimethylaminobenzaldehyde, isoamyl alcohol and HCl) was added. A formation of a cherry red or purple red ring at the interface of the broth culture and the reagent indicated the indole production from tryptophan by the sample. 2. Methyl red test (MR test): To an aliquot of 5 mL of sterile MRVP broth (peptone 7 g, glucose 5 g, potassium phosphate 5 g, pH 6.9), the test culture was inoculated and incubated for 48 h at 37 ℃. To this culture, 5 drops of methyl red was added as indicator. If the total solution turns red, the test is positive for the formation of organic acids as end products. 3. Voges-Proskauer test (VP test): To an aliquot of 5 mL of sterile MRVP broth, the test culture was inoculated and incubated for 48 h at 37 ℃. To this culture tube, 10 drops of VP I reagent (5% α-napthol dissolved in absolute alcohol) and 2-3 drops of VP II reagent (40% KOH solution) was added and the mixture was allowed to stand for 15-20 min for the reaction to complete. From an appearance of red colour, it was inferred a positive result *i.e.*, production of a neutral product, acetoin from the fermentation of glucose by the organism, and alternately yellow colour production inferred the negative result. 4. Citrate test: The test culture was inoculated onto a slant of Simon citrate agar was incubated for 48 h at 37 ℃. The change of color of agar from green to blue indicated that organism used citrate as the sole source of carbon. 5. Urease test: The test organism was inoculated to a slant of Christensen's urea agar (peptone, glucose, sodium chloride, mono-potassium phosphate, urea, phenol red, distilled water, and at pH 6.8). The hydrolysis of urea yielding ammonia increases the pH that changes the colour of the medium from off-white to pink/orange which infered a positive result. 6. Triple-sugar-iron test: Two or 3 drops of test broth culture was inoculated on triple-sugar-iron agar slant and then a stab was made up to the butt of the slant. The tube was incubated for 37 ℃ for 48 h; the black color appearance indicated H_2S production. 7. Nitrate test: An aliquot of 5 mL of nitrite broth was inoculated with 1 drop of 24 h old broth test culture and was incubated for 48 h at 37 ℃.

The development of red colour within 30 s of adding a few drops equal volume 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) inferred positive results. No colour change suggested negative results.

2.5. Antibiotic sensitivity test

All bacterial strains were subjected to antibiotic sensitivity test by Kirby-Bauer's method, using a 4 mm thick Mueller-Hinton agar (MHA) medium[15]. An aliquot of 0.1 mL of 38.9-41.5 $\times 10^{10}$ cfu/mL (0.5 McFarland equivalents), approximately from an exponentially growing culture was spread on to agar for development of lawn of any strain of bacteria. Further on lawn-agar of each plate, 6 high potency antibiotic-discs (Himedia, Mumbai) of 12 prescribed antibiotics for each bacterial isolate were placed at equal distances from one another. Plates were incubated for 30 min at 37 ℃ in a BOD incubator (Remi CIM -12s) for 18 h at 37 ℃, after which diameter values of zones of inhibition were measured. The antibiotics used for each pathogenic bacterium were recommended ones prescribed for control, according to 'National Committee for Culture Laboratory Standards' guidelines.

2.6. Antibacterial activity test

For monitoring antibacterial activity by the agar-well diffusion method[16], bacterial lawn was prepared. Wells were punched for 6 mm deep in 30 min old bacterial lawn 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 18-24 h.

Antibacterial activities were evaluated by measuring

the diameter values of zones of inhibition; monitoring experiment of each solvent extract was conducted thrice and results of the third repetition are presented. It was confirmed that 10% DMSO had no inhibitory effect on any bacterium.

3. Results

The list of plants used along with tribal information gathered for their uses are given in Table 1. Water and ethanolic extracts of all plants (leaves and/or barks) were prepared; weights of the concentrated of alcoholic extracts sticky masses are given in Table 2. It is discernible that different plants yielded different amounts of phytoextracts.

Specific bacterial colonies were isolated from particular plates, depending upon gross colony morphology of pure cultures (Table 3). Based on the inherent logic of biochemical tests, isolated pathogenic strains were

Table 1.
Ethno-medicinal report of 20 medicinal plants.

Sl. no	Plant name with voucher specimen number	Family	Vernacular name	Parts used	Ethno-medicinal uses
1	*Argemone mexicana* Linn KLDJ/076	Papaveraceae	Bada gokhura	Leaf	Scabies, eye troubles, menorrhea, spermatorrhoea, jaundice, wound infections. A tea spoon of latex is given every day during jaundice.
2	*Argyriea speciosa* Sweet KLDJ/093	Convolvulaceae	Brudha daraka	Leaf	Rheumatism. During piles, corm paste is fried in ghee (cow-milk fat), and served as tablets. Three tablets are taken for 30-40 days.
3	*Anthocephalus cadamba* Miq. KLDJ/150	Rubiaceae	Kadamba	Leaf	For treatment of urinary tract infections and biliousness bark is used.
4	*Aspidoptrerys tomentosa* Roxb. KLDJ/184	Malpighiaceae	Altilaha/Katilaha	Leaf	Roots are boiled in til oil (*Sesamum indicum*) and are applied in treatment of eczema.
5	*Butea monosperma* Lam.KLDJ/253	Fabaceae	Palasa	Leaf	Diarrhoea. For blood pressure the flower is soaked in warm water for overnight, filtered and taken in empty stomach for a month. One table spoon of table decoction is given for 3 days after 5th day of menstruation for conception.
6	*Calotropis procera* Ait.KLDJ/076	Asclepiadaceae	Arakha	Leaf	Leprosy, stomach disorders, rheumatism, headache, flatulence, stomachache. Small incision is made at the same side of forehead and a drop of latex is applied over the cut for migraine.
7	*Cassia fistula* Linn. KLDJ/076	Caesalpiniaceae	Sunari	Leaf	Amenorrhea, skin disease, constipation. Bark paste applied locally for healing for wounds and raw juice is taken orally to for curing dysentery.
8	*Celastrus paniculatus* Willd.KLDJ/076	Celastraceae	Pengulai	Leaf	Boils, leucorrhoea, piles, spermatorrhoea. Seeds are pounded in stone and applied on the skin to cure ringworm infection, scabies and eczema.
9	*Cissus quadrangularis* Linn. KLDJ/076	Vitaceae	Hadajoda	Leaf	Dyspepsia, indigestion, joining of fractured bones and swellings. Powdered root is used for specific fracture. Oral administration of plants has an analgesic effect on animals.
10	*Cleistanthus collinus* Roxb.KLDJ/076	Euphorbiaceae	Karada	Leaf	Soft drink prepared from little amount leaf leachate, roots and fruits are used for skin.
11	*Dalbergia paniculata* Roxb.KLDJ/076	Fabaceae	Dhoben	Leaf	For dysentery, 20-30 ml of bark juice is taken once, if needed 2nd dose is given on alternate day.
12	*Diospyrous melanoxylon* Roxb. KLDJ/076	Ebenaceae	Kendu	Leaf	Diuretic, laxative, dried flowers are used against UTI infections and skin diseases. Decoction of the bark is used in diarrhea and dyspepsia.
13	*Elephantopus scaber* Linn.KLDJ/076	Asteraceae	Mayura chulika	Leaf	Headache, pyorrhea, diarrhea. Root paste is mixed with raw milk and taken orally as laxative. Root paste is taken with rice water in empty stomach for 3-4 days for irregular menstruation.
14	*Ficus glomerata* Roxb.KLDJ/076	Moraceae	Dimiri; Dumer	Leaf	Small pieces of unripe fruit fried in ghee (animal fat) are given in small amount 3-5 times daily to cure sexual disability and nocturnal ejaculation.
15	*Nicotiana tobaccum* Linn.KLDJ/076	Solanaceae	Dhingia	Leaf	Scabies. Processed and dried leaf powder in water for 10-15 minutes and two drops of water is administered into the infected eyes.
16	*Oroxylum indicum* Vent.KLDJ/076	Bignoniaceae	Phapen	Leaf, Bark	Scabies, leprosy, diarrhea, pyorrhea. 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. Water is boiled with bark and used for bathing.
17	*Pterocarpus santalinus* Linn. KLDJ/076	Fabaceae	Rakta-chandan	Leaf,Bark	Blood purifier, diuretic. During chest pain and TB, paste is made from the wood and applied on the chest. Also solution with water is made and taken for a month orally.
18	*Strychnos noxvomica* Linn.KLDjl/076	Loganiaceae	Kochila	Leaf	Acute diarrhoea. Mixed with lemon juice and made into pills and taken orally during dysentery, arthritis, rheumatism and piles.
19	*Terminalia alata* Hexne ex Roth. KLDJ/368	Combretaceae	Sahaj	Leaf	For epilepsy, aliquots of 20-30 ml of bark is given daily for a month or till symptoms disappear.
20	*Woodfordia fruticosa* Linn.KLDJ/376	Lythraceae	Dhatiki	Leaf	Astringent, menorrhagia, leprosy, burning sensation, haemoptisis.

Table 2.
Amount of sticky mass of phytoextracts (5 g powders in 80 mL ethanol) at recovery from rotary evaporator and stock solution prepared by adding aliquots of 1 mL of 10% DMSO.

Serial no	Plant name	Weight of ethanolic extract (mg)	Stock concentration (mg/mL)
1	*Argemone mexicana*	0.07	70
2	*Argyriea speciosa*	0.09	90
3	*Anthocephalus cadamba*	0.08	80
4	*Aspidoptrerys tomentosa*	0.12	120
5	*Butea monosperma*	0.09	90
6	*Calotropis procera*	0.17	170
7	*Cassia fistula*	0.20	200
8	*Celastrus paniculatus*	0.12	120
9	*Cissus quadrangularis*	0.22	220
10	*Cleistanthus collinus*	0.12	210
11	*Dalbergia paniculata*	0.12	120
12	*Diospyrous melanoxylon*	0.28	280
13	*Elephantopus scaber*	0.26	260
14	*Ficus glomerata*	0.19	190
15	*Nicotiana tobaccum*	0.19	190
16	*Oroxylum indicum*	0.07	70
17	*Pterocarpus santalinus*	0.18	180
18	*Strychnos nux-vomica*	0.23	230
19	*Terminalia alata*	0.17	170
20	*Woodfordia fruticosa*	0.27	270

Table 3.
Media used for isolation and maintenance of bacteria from clinical samples and their colony characteristics.

Sl. no	Bacterium	Agar media	Colony morphology
1	*S. aureus*	Blood agar	Medium to large, smooth, entire, slightly raised, creamy yellow, with green/â hemolytic colonies
		Nutrient agar	As above without hemolytic activity
2	*Acinetobacter* sp.	Nutrient agar	Colourless smooth, opaque, raised and pinpoint colonies
		MacConkey agar	Colourless smooth, opaque, raised, NLF colonies
3	*C. freundii*	MacConkey agar	Late LF colonies light pink after 48 h
4	*C. violeceum*	Nutrient agar	Round smooth, and dark purple colour colonies
5	*E. coli*	Nutrient agar	Flat dry, irregular colonies
		MacConkey agar	LF , flat dry pink, irregular colonies
		EMB agar	Flat dry, irregular colonies, with metallic green colour.
6	*Klebsiella* sp.	MacConkey agar	LF, pink, mucoid colonies
7	*Proteus* sp.	MacConkey agar	NLF, colourless foul smelling colonies
8	*P. aeruginosa*	Nutrient agar	Large, irregular opaque colonies, with bluish green pigment
9	*S. typhi*	MacConkey agar	NLF, colourless colonies
		XLD agar	Red colour, pinpoint colonies with black center
10	*V. cholerae*	TCBS agar	Smooth, opaque, yellow colour colonies

Note: EMB: eosine-methylene-blue agar; SS: salmonella-shigella agar; TCBS: thio-sulfate-citrate-bile-salts-sucrose agar; XLD: xylose-lysine-deoxylcholate agar. LF: lactose fermenting; NLF: non-lactose fermenting.

confirmed and assigned to a taxon (Table 4). For example, *S. aureus* cells were Gram-positive cocci with catalase, coagulase and oxidase activities, which help in the biochemical identification of this bacterium along with the standard strain. Rest other bacteria were too assigned to the respective taxa using morphological and biochemical identifying features (Tables 3 & 4).

Both cold water and ethanolic extracts were tested against ten MDR bacteria by the agar-cup method; values of zones of inhibition due to both types of extracts are given in Table 6. Ethanolic extracts of most plants were more effective as antibacterial agents than the corresponding water extracts for almost all bacteria (Table 6). For example, *S. aureus* was found sensitive to 17 ethanolic plant extracts, whereas only 10 water extracts could control it *in vitro*. Similar values can be ascertained for rest other bacteria (Table 6). Conversely, both ethanolic extracts and water extracts of *Argemone mexicana* were found to control *S. aureus, Acinetobacter, C. freundii* and *P. aeruginosa*; but *C. violeceum, E. coli* and *Klebsiella sp.* were not controlled by either extract of *A. mexicana*; on the other hand, *Proteus sp., S. typhi* and *V. cholerae* were controlled by its ethanolic extract (Table 6).

Ethanolic extracts of *Celastrus paniculatus, Butea monosperma*, and water extracts of *C. paniculatus, B. monosperma, Woodfordia fruticosa* and *Elephantopus scaber* were found to be effective against MDR *S. aureus*, whereas ethanolic extracts of *Diospyrous melanoxylon* was only moderately effective (Table 7).

Table 4.
Summary of results of biochemical tests of ten pathogenic bacteria.

Bacterium (MDR strains)	Gram stain/shape	Catalase	Oxidase	Indole	Methylred	Voges-Proskauer	Citrate	Urease	TSI	Nitrate reduction
S. aureus	+ve cocci	+ve	+ve	nd	nd	nd	nd	+ve	nd	nd
Acinetobacter sp.	-veCocco-bacilli	+ve	-ve	nd	nd	nd	nd	nd	nd	-ve
C. freundii	-ve rods	+ve	nd	nd	+ve	-ve	+ve	-ve	A/G	nd
C. violeceum	-ve rods	nd	+ve	+ve orange	nd	-ve	nd	nd	nd	-ve
E. coli	-ve rods	+ve	-ve	+ve	+ve	-ve	-ve	-ve	A/G	+ve
Klebsiella sp.	-ve rods	+ve	-ve	-ve	-ve	+ve	+ve	+ve	A/GH2S	+ve
Proteus sp.	-ve rods	+ve	-ve	-ve	+ve	-ve	-ve	+ve	A/GH2S	+ve
P. aeruginosa	-ve rods	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
S. typhi	-ve rods	+ve	-ve	-ve	+ve	-ve	+ve	-ve	A/GH2S	+ve
V. cholerae	-ve rods	+ve	+ve	+ve	-ve	-ve	nd	-ve	nd	+ve

Note: See the text for details of biochemical tests. 'nd' = not done. *S. aureus* is identified as Gram +ve cocci. It secretes enzymes, catalase and coagulase, which help in the biochemical identification of this bacterium. *E. coli* is identified as Gram -ve motile bacilli, which respond to catalase, indole-test, Methyl red, VP-test and nitrate reduction tests, used for its identification. *S. typhi* is Gram -ve motile bacilli, which respond to catalase, MR, citrate and nitrate tests. Similarly, *V. cholerae* is Gram -ve, comma shaped highly motile cells, which respond to catalase, oxidase, indole and nitrate reduction tests. A/G = acid and gas production.

Table 5.
Antibiogram of the ten bacteria isolated from patients sample. Values as zone of inhibition in mm due to antibiotics disks.

	S. aureus	*Acinetobacter* sp.	*C. freundii*	*C. violeceum*	*E. coli*	*Klebsiella* sp.	*Proteus* sp.	*P. aeruginosa*	*S. typhi*	*V. cholerae*
Nx	R	27	17	32	R	R	18	R	-	22
Nf	20	18	16	30	15	17	11	R	16	22
Ak	12	21	21	21	15	19	15	16	19	R
Ce	R	21	R	16	R	R	13	R	-	-
I	R	R	R	R	R	R	R	20	-	20
Pt	R	R	R	R	R	R	R	12	-	-
Gf	R	30	R	32	16	18	17	16	14	-
Of	R	25	R	31	R	R	13	R	-	-
Nt	14	19	R	21	15	15	R	R	18	-
Ac	R	16	R	R	R	R	R	R	-	-
Va	R	nd	nd	nd	nd	nd	nd	nd	-	-
A	-	-	-	-	-	-	-	-	R	R
G	-	-	-	-	-	-	-	-	R	24
C	-	-	-	-	-	-	-	-	21	-
Co	-	-	-	-	-	-	-	-	19	R
Na	-	-	-	-	-	-	-	-	R	21
Ci	-	-	-	-	-	-	-	-	R	-
E	-	-	-	-	-	-	-	-	-	24
St	-	-	-	-	-	-	-	-	-	23
Ne	-	-	-	-	-	-	-	-	-	22

Note: Antibiotic-disks: A: ampicillin 30 mcg; C: chloramphenicol 30; Ak: amikacin 30; Ac: amoxyclave 30; Ce: cefotaxime 30; Co: Co-trimoxozole 30; E: erythromycin 30; G: gentamicin 30; Gf: gatifloxacin 30; I: imipenem 10; Na: nalidixic acid 30; Ne: neomycin 30; Nf: nitrofurantoin 300; Nt: netilmicin 30; Nx: norfloxacin 300; Of: ofloxacin 5; Pt: piperacillin & tazobactam 100 &10; St: streptomycin 30; Va: vancomycin 30 mcg/disk. For S. aureus, oxacillin 1mcg/disk was used and lawns had no inhibition zone. 'R' indicates resistance of the bacterium. Data of the second repeated experiment are presented. All values are mean of duplicate readings. Antibiotics used were according to 'National Committee for Culture Laboratory Standards' guidelines. Antibiotic-disks were different for *S. typhi* and *V. cholerae*.

Table 6.
Result of screening of selected medicinal plants by the agar cup method.

Bacteria	Zone of inhibition by twenty plant extracts (mm)																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16 leaf	16 bark	17leaf	17bark	18	19	20
S. aureus MRSA	14(11)	14(-)	10(-)	12(10)	26(18)	15(-)	11(8)	29(21)	15(-)	11(12)	-(-)	21(6)	20(17)	11(-)	14(12)	-(-)	17(14)	12(-)	12(-)	14(11)	-(-)	15(18)
Acinetobacter sp.	12(12)	-(-)	10(-)	18(13)	-(-)	-(-)	-(-)	-(-)	-(-)	-(-)	17(14)	-(-)	11(-)	-(-)	-(-)	12(-)	12(-)	14(-)	16(12)	14(-)	12(10)	10(-)
C. freundii	12(12)	-(-)	-(-)	-(-)	-(-)	14(-)	12(-)	13(13)	14(-)	14(-)	12(-)	-(-)	18(14)	14(12)	12(-)	16(12)	16(12)	11(-)	-(-)	-(-)	-(-)	-(-)
C. violeceum	-(-)	14(-)	14(13)	-(-)	23(18)	16(11)	-(-)	18(16)	16(11)	14(-)	14(-)	-(-)	16(-)	-(-)	-(-)	-(-)	16(-)	14(-)	-(-)	-(-)	12(-)	16(-)
E. coli	-(-)	12(12)	17(11)	14(10)	23(16)	18(-)	2016	18(-)	18(-)	13(-)	-(-)	8(6)	19(12)	-(-)	12(-)	16(11)	16(12)	12(-)	14(11)	-(-)	12(-)	-(-)
Klebsiella sp.	-(-)	12(-)	12(-)	12(-)	12(-)	-(-)	14(-)	13(-)	-(-)	10(-)	12(-)	-(-)	-(-)	-(-)	12(-)	-(-)	11(-)	14(11)	12(-)	16(11)	-(-)	-(-)
Proteus sp.	12(-)	12(-)	13(20)	-(-)	16(-)	10(-)	-(-)	-(-)	10(-)	-(-)	12(-)	-(-)	21(-)	12(-)	-(-)	-(-)	10(-)	12(-)	14(14)	-(-)	14(10)	11(-)
P. aeruginosa	14(18)	-(-)	15(-)	1411	-(-)	-(-)	11(8)	15(-)	-(-)	12(-)	17(14)	21(6)	-(-)	18(12)	15(-)	-(-)	-(-)	-(-)	12(-)	13(13)	-(-)	-(-)
S. typhi	13(-)	18(12)	2(-)	14(12)	17(13)	17(-)	14(12)	12(-)	17(-)	11(-)	14(10)	22(-)	12(-)	-(-)	-(-)	12(-)	14(-)	-(-)	18(13)	11(-)	12(15)	
V. cholerae	12(-)	13(-)	11(-)	13(11)	-(-)	-(-)	12(-)	-(-)	-(-)	10(-)	10(10)	14(6)	-(-)	14(10)	13(-)	13(-)	19(14)	-(-)	12(12)	13(13)	15(-)	-(11)

Note: Numbers 1 to 20 are serial numbers of plants given in Table 1; Except *S. aureus*, all bacteria are Gram -ve. Upper row of values are measurements of zone of inhibition due to ethanol-extracts and lower values in parenthesis ( ) are due to water-extracts. Data of the third repeated experiment are presented. All values are mean of triplicate readings. "-" sign denotes no activity.

Table 7.
Summary of antibacterial activity of ethanolic and water extracts of plants against pathogenic bacteria.

Sl. no	Bacterium	Effective ethanolic extracts	Effective water extracts
1	*S. aureus*	1,2,3,4,5,6,7,8,9,10,12,13,14,15,16(bark),17,18, 20	1,4,5,7,8,10,12,13,15,16 (bark),18,20
2	*Acinetobacter* sp.	1,3,4,11,12,16(leaf,bark),17(leaf,bark),18,19, 20	1,4,11,17(bark),19
3	*C. freundii*	1,6,7,8,9,10,11,13,14,15,16(leaf,bark),17(leaf)	1,8,13,14,16(leaf),16(bark)
4	*C. violeceum*	2,3,5,8,9,10,11,13,16(bark),17(leaf),19,20	3,5,6,8,9
5	*E. coli*	2,3,4,5,6,7,8,9,10,15,16(leaf,bark),17(leaf,bark),19	2,3,4,5,7,12,13,16(leaf,bark),17(bark)
6	*Klebsiella* sp.	2,3,4,5,7,8,10,11,15,16(bark),17(leaf,bark),18	17(leaf), 18
7	*Proteus* sp.	1,2,3,5,6,9,11,13,14,16(bark),17(leaf,bark),19,20	3,17(bark), 19
8	*P. aeruginosa*	1,3,4,7,8,10,11,12,14,15,17(bark),18	1,4,7,11,12,14,18
9	*S. typhi*	1,2,3,4,5,6,7,8,9,10,11,12,13,16(bark),17(bark),18.19.20	2,4,5,7,11,12,18,20
10	*V. cholerae*	1,2,3,4,7,9,10,11,14,15,16(leaf,bark),17,(bark),18,19	11,12,14,16(bark),17(bark),18,20

Note: the numbers in column 2 and 3 represents name of plants listed in Table 1.

Both ethanolic and water extracts of *Aspidoptrerys tomentosa, Dalbergia paniculata, Pterocarpus santalinus* (bark) were found to be effective against Acinetobacter. For *C. freundii*, ethanolic extracts *E. scaber* and *Oroxylum indicum* (leaf and bark) were effective, whereas that of *C. paniculatus*, *A. mexicana* and *Ficus glomerata* had moderate antibacterial activity. Both ethanolic and water extracts of *B. monosperma* and *C. paniculatus* were highly effective against *C. violeceum*, whereas those of *Anthocephalus cadamba*, *Calotropis procera* and *Cissus quadrangularis* were moderately effective.

For *E. coli,* water and ethanolic extracts of *B. monosperma*, *Cassia fistula* registered good antibacterial activities, whereas *Terminalia alata*, *A. cadamba, O. indicum* (leaf and bark) and *P. santalinus* (bark) showed moderate antibacterial activity. Ethanolic extracts of *C. paniculatus*, *C. quadrangularis* showed good antibacterial activities for *E. coli*. With Klebsiella, ethanolic extracts of *P. santalinus* (leaf) and *Strychnos nux-vomica* recorded moderate antibacterial activities. For *Proteus* sp., ethanolic extract of *E. scaber* and water extract of *A. cadamba* were highly effective; similarly, ethanolic extracts of *B. monosperma, P. santalinus* (bark), *T. alata* and water extract of *P. santalinus* (bark) were moderately sensitive.

P. aeruginosa was found to be highly sensitive to ethanolic extracts of *D. melanoxylon* and *F. glomerata* and water extract of *A. mexicana*; and ethanolic extract of *A. mexicana*, *A. cadamba*, Aspidoptrerys tomentosa, *C. paniculatus*, Nicotiana tobaccum, and water extracts of *D. paniculata* and *S. nux-vomica* showed moderate antibacterial activity.

Both water and ethanolic extracts of *D. melanoxylon* were highly active against *S. typhi*, whereas water and ethanolic extracts of *Argyriea speciosa*, *A. tomentosa, B. monosperma, C. procera, C. fistula, D. paniculata, S. nux-vomica* and *W. fruticosa* were found to be moderately effective. *V. cholerae* was found sensitive to ethanolic extracts of *D. melanoxylon*, *F. glomerata, O. indicum* (bark), *T. alata* and water extracts of *O. indicum* and *S. nux-vomica* (Table 7).

4. Discussion

Multiple antibiotic resistances in pathogenic bacteria has become a serious matter of concern, as reasons like natural evolutionary modes of obtaining both intrinsic and extrinsic resistance patterns[17] are blamed for rapid and colossal occurrences of MDR bacteria. Pathogenic bacteria evolve new strains gaining resistance to recently used antibiotics and drugs, an event which repeats by itself, and in last few decades there have been an increase in the occurrence of MDR pathogens worldwide. Concomitant to search for new generation of drugs for increased MDR pathogenic bacteria, continual efforts for search of control agents from plants and related sources have been undertaken[18,19].

This study is unique compared to most other works in the field of monitoring antimicrobial activities of medicinal plants, as herein drug-sensitive bacteria obtained from type culture collection centers as well as MDR strains of pathogens obtained directly from patients are used. This study is a pure academic work, as it lacks toxicity studies with animals; nonetheless MDR human pathogens were directly used for monitoring antimicrobial activities of 20 plants, with ethno-botanical information against infectious elements recorded from aborigine Indians.

Of the two polar solvents ethanol and water, the former was found to be invariably a better solvent for almost all plants used. Reports published so far do not generally consider importance of solvents during extraction, notwithstanding routine uses of three or four organic solvents like methanol, butanol, propanol and chloroform.

Screening with this large number of plants would lead to a conclusion that non-edible plant species were suitable for antimicrobial activities against pathogenic bacteria. Furthermore, most of the bacteria studied herein are potential enough to cause havoc in the management of patients, because of MDR strains, to extents that would warrant alternative drug therapy. For example, *V. cholerae* is resistance to ampicillin, amikacin and co-trimoxazole, for which the earlier two antibiotics are never applied to

V. cholerae. It could be due to the wide spread genetic recombination methods operative in nature, which may be one of many causes of multiple resistance in all pathogenic bacteria. Nosocomial infections of pathogenic bacteria including MDR *S. aureus* widespread in a hospital referred herein could be facilitating for the general exchange of genetic materials *i.e.*, resistance factors[20].

The genus, *Staphylococcus* includes pathogenic organisms of which, *S. aureus* is frequently isolated from clinical samples. This species has overcome the control from most modern therapeutic agents; eventually its control has become the most hazardous. Further, β-lactamase activity of *S. aureus* has caused resistance to most penicillin derivatives including the oxacillin and now is identified as MRSA, which has become the major the cause of nosocomial infections worldwide, including India[21].

An Indian report describes work done on *in vitro* control of MDR and ATCC strains of *E. coli*, *K. pneumoniae*, *Streptococcus mutans*, *S. bovis*, *Enterococcus faecalis*, *P. aeruginosa*, *S. aureus*, *Salmonella typhimurium* using ethanolic extracts of five plants, *Acacia nilotica*, *Syzygium aromaticum* and *Cinnamomum zeylanicum*, *T. arjuna* and *Eucalyptus globules*. The most potent antimicrobial plant was *A. nilotica* (with a MIC range of 9.75-31.3 μg/mL)[22].

A crude methanol extract of *Garcinia nigrolineata* had antibacterial activity against MRSA. An ethanol extract of *Garcinia kola* was tested against MRSA; MIC value of the extract was 0.08-1.8 mg/mL, while the MBC value ranged from 0.135 to 4.2 mg/mL. Thus, *G. kola* was recorded to be strongly active against MRSA[23].

A 50% ethanol extract of the dried fruits (with chebulagic acid, chebulinic acid, corilagin, gallic acid, punicalagin, terchebulin, and terminalic acid) of *Terminalia chebula* (local Haritaki) inhibited the growth of MRSA, with a MIC value of 31.3 mg/mL. This plant, native to India has been used in traditional medicines to treat respiratory tract infections[23].

The tea tree (*Melaleuca alternifolia*) oil was reported to control 66 clinical isolates of *S. aureus*; of the isolates tested, 34 were MRSA and 32 were mupirocin-resistant *S. aureus*; the MIC and the MBC values were 0.25% and 0.50% diluted oil, respectively. Moreover, some of the naturally occurring compounds in the oil, including 1, 8-cineol (4.5%-16.5%), terpinen-4-01 (29%-45%), y-terpinene (10%-28%) and a-terpineol (2.7%-13.0%), were recorded to show a reduced growth pattern of MDR *S. aureus*, *in vitro*, without any bacterial resistance to the oil even at a concentration of 2.5% (v/v)[23].

An alkaloid of Hypericum perforatum (St. John's wort, a traditional folk medicine for wounds), hyperforin at a concentration of 1 mg/mL has been recorded to inhibit the growth of MRSA. Berberine, obtained from a number of plants including Coptis chinensis, Berberis vulgaris and Hydrastis canadensis was also active against MRSA *in vitro*. Berberine inhibited the growth of *S. aureus*, with a MIC value of 25.0 mg/mL. Sub-inhibitory concentrations of berberine were potentiated by the flavones chrysosplenol-D and chrysoplenetin from *Artemisia annua*. This was due to the inhibition of a MDR pump in MRSA, an intrinsic mechanism of multiple drug resistance. This is an example of synergistic effects of phytochemicals against MRSA[24]. The antimicrobial activity of a berberine-containing extract from rhizome of *Coptis chinensis* was found effective against *S. aureus* ATCC 6538 (wild drug-sensitive strain), with a MIC value of 8.7 mg/mL[23].

Crude extracts of 10 Brazilian pants were screened for antibacterial activity of 7 clinical MDR microorganisms utilizing as control ATCC strains. Ethanol extracts of plants, *Geissospermum argenteum*, *Uncaria guianensis*, *Brosimum acutifolium*, *Copaifera reticulate*, *Licania macrophylla*, *Ptycopetalum olacoides* and *Dalbergia subcymosa* were effective against MDR *S. aureus* and MDR *P. aeruginosa* (the urinary tract pathogen), and the *S. aureus* ATCC strain 6538[25].

Today, certain Gram-negative bacteria resistant to all the major classes of antibiotics have been emerged and these are cited as pandrug-resistant bacteria; such pathogenic strains (more spectacularly, *P. aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*) cause utmost comorbidities and frequent immature mortality.

It would be prudent to take recourse to plants (and animals) systematically for the holistic control of MDR bacteria as complementary medicines, or else are we heading to a post-antibiotic era with the 'butterfly' theory of chaos in issues of healthcare and management of infections[26].

Effective *in vitro* control of MDR strains of *Acinetobacter* sp., *C. freundii*, *Proteus* sp. and *P. aeruginosa*, the most potential urinary tract infection causing organisms by plant extracts of all major plant used herein is recorded. MDR *C. violaceum* isolated from skin lesions was found to be resistant to imipenem, piperacillin-tazobactam and amoxyclav and was found sensitive to 13 plant extracts. Enteropathogenic bacteria, *E. coli*, *S. typhi*, *Klebsiella* sp. and *V. cholerae* were also found to be well controlled by all plant extracts used. The armada of chemotherapeutic drugs and dovetailed antibiotics against MDR pathogens plausibly could marvel and shudder the present resistant infection-problem, if the principle of synergism with suitable phytochemicals as complementary medicine in a revised therapeutic module is adopted with adept in the crusade MDR pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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