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Surveillance of multidrug resistance of 10 enteropathogens in a teaching hospital and *in vitro* efficacy of 25 ethnomedicinal plants used by an Indian aborigine

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ABSTRACT

Objective: To have an antibiogram of hospital acquired (HA) and community acquired (CA) enteropathogens against 16 antibiotics to assess the infection dynamics for plausible help to the antimicrobial stewardship. To check extracts of 25 lesser-known plants used by an Indian aborigine, for antimicrobial efficacy *in vitro* and as complementary and alternate medicines against resistant pathogens. **Methods:** Ten strains of enteric bacteria (*Enterobacter aerogenes, Escherichia coli, Klebsiella* sp., *Salmonella paratyphi, S. typhi, Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei and Vibrio cholerae*) were isolated from clinical samples in 6 months and their antibiotic sensitivity was assessed by the disc-diffusion method. Concentrated aqueous and ethanolic extracts of leaves and barks of plants were used for monitoring their antibacterial potencies, by the agar-well diffusion method. **Results:** Isolated bacterial strains were invariably multidrug resistant (MDR). *E. coli* was the most frequently isolated organism from HA and CA samples, followed next by *Klebsiella* sp. From the surveillance, it was evident that the distribution of MDR strains of each was more in HA than CA isolates. Aqueous and ethanolic extracts of *Aegle marmelos, Azadirachta indica, Cassia fistula, Holarrhena antidysenterica, Salvadora persica* and *Terminalia arjuna* were highly effective against the all isolated enteropathogenic strains. From the preliminary phytochemical analysis, it was confirmed that both extracts of *A. indica, T. arjuna* and *T. alata* contained all the detected phytochemicals (alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids), which plausibly attributed to their significant antibacterial activity. **Conclusions:** Phytoextracts were highly effective against the all enteropathogenic bacterial isolates, *in vitro*. These 25 plants could be used further for the isolation of pure compounds for use as complementary medicines.

1. Introduction

Bacterial pollution of inland waters has become one of the most important public health concerns worldwide, and in India it is graver than imagined, because of the absence of a developed sewage disposal system, in villages and towns at least [1, 2]. Further, it is estimated that there were 93.06 million approximately disadvantaged slum-dwellers in cities without a hygienic management of domestic sewage, in India [3]. More often, hospital sewage/wastes too are badly disposed [1]; consequently, contamination of inland waters by enteric bacteria is the commonplace of infections [4, 5]. Further, animal husbandry establishments contribute coliform and many enteropathogenic bacteria to inland waters in many countries, because of the lack of scientific disposal of organic farm wastes, as compost. Eventually, sporadic outbreaks of enteropathogenic bacteria including cholera have been frequently precipitated in many countries [6, 7], and the under-5 mortality in children from diarrhoea has been surfaced in several developing countries [7, 8], creating an uproar in community health; and when those bacteria are reported independently by almost all countries as multidrug resistant (MDR) [9, 10], there would be outraging commotion with adults even in public health as documented from Malaysia [14]. Thus, the hygienic totem pole of drinking water system as well as the clinical world, in general, gets

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challenged.

MDR *Escherichia coli* have been reported from our laboratory as contaminants of community and hospital setups [11]. Unfortunately, there is stringent antibiotic policy nowhere; consequently, antibiotics are misutilized, leading to the emergence of MDR pathogens. Meta-analysis of nosocomial infections of hospitals have been published worldwide, mainly estimating morbidity, mortality and associated costs of the most Gram-positive bacteria and *E. coli* among the Gram-negative ones [9,13], but attempts of surveillance of enteropathogenic bacteria were limited [8-14]. Nevertheless, mortality rate in developing countries reaches at times to saturnine heights due to enteropathogens [1]. So, the surveillance of commonly isolated enteropathogens had been initiated to have the local estimations of the problem.

Herein, antibiogram of 10 enteropathogens monitored against 16 antibiotics were recorded to examine the infection status of two obvious sources, hospital and community. Drug targeting with new antimicrobials are the main stream work of apothecary against all MDR pathogens, but the advantage of crude phytoextracts is that no pathogen can override the plethora of natural non-microbial phytochemicals. As pharmaceuticals are the central to patient care, these results coupled with antibiograms of a cohort of MDR enteropathogens isolated from clinical samples, and data on the efficacy of extracts of 25 lesser-known plants could help the pharmacy world for the search of new drugs with finesse to circumvent these ferocious pathogens. The use of crude phytochemicals may appear prosaic, but information lent from ethnobotany is age-tested and necessary to be evaluated and embroiled for reducing the cataclysmic guiles of MDR enteropathogens. Cognitively, host toxicity due to these plants should be less as each plant has a history of ethnomedicinal uses in the odyssey of Odishan tribal culture down the ages, that being never recorded before.

2. Materials and methods

2.1. Survey work

Information of plants were obtained from the Kandha tribe at hills of Eastern range of mountains of India, in the district Kalahandi, Odisha in February 2010. About 50 respondents of 20 hamlets were interviewed in a forest patch and the recorded information was documented (Table 1). The snowball method of survey and sampling was used.

2.2. Preparation of plant extracts

Collected mature leaves/barks of plants were crushed to powders. A lot of 5 g of powder was dissolved in an aliquot of 25 mL of double distilled water and was sterilized for 30 min, before incubation at 4°C for 72 h with intermittent stirring. These steps were repeated for each plant sample. Aqueous 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 usual hand-shakings and was filtered. The ethanolic-filtrate was concentrated in a rotary evaporator at 40°C, 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°C until further use. Preliminary phytochemical analyses were done, as previously described [15].

2.3. Collection of bacterial strains

This is a philanthropic teaching hospital. Details of collection of bacteria are presented in Table 2; a total of 652 isolates, i.e., 380 isolates from hospital acquired (HA) and 272 isolates from community acquired (CA) samples were collected. Ten enteropathogens (*Enterobacter aerogenes, Salmonella paratyphi, Shigella boydii, S. dysenteriae, S. flexneri, S. sonnei, Vibrio cholerae*) were isolated during a span of 6 months. Biochemical identifications of isolated bacterial strains are described previously [15]. Bacterial strains were ascertained to taxa with results of biochemical tests recorded in Table 3. All bacterial strains were subjected to antibiotic sensitivity tests by the disc diffusion/ Kirby-Bauer's method, described in detail previously [15]. Sixteen antibiotics of 5 different groups were used for determining the antibiotic sensitivity patterns of isolated bacteria. Antibacterial activity tests by agar-well diffusion method using one strain from each bacterial species for monitoring antibacterial potentiality of plants extracts were done, as described [15, 16].

3. Results

Ethnomedicinal information on 25 plants documented in Table1 was too recorded along with details of modalities about crude extracts as medicine for many ailments. Most of these plants were lesser-known/ non-common and are in the use for infectious diseases, by aborigines.

E. aerogenes was identified basing on its colony characteristics on blood agar and MacConkey agar along with the results of 9 biochemical tests. White coloured convex colonies were formed on blood agar with γ-haemolysis (Figure 1), and pink coloured colonies developed because of lactose fermentation (LF) on MacConkey agar (Table 2). Further, it was found positive to catalase, VP, citrate and nitrate reduction tests and negative to oxidase, indole, methyl-red and urease tests. Shown in Table 3, with the TSI test the bacterium was recorded to produce only acid, but no gas. Similarly, the rest 9 bacterial isolates were identified basing on their colony characteristics on suitable media and biochemical test results, as well (Tables 2 and 3).

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 ophthalmia.
2	*Anthocephalus cadamba* (Roxb.) Miq.	Rubiaceae	Kadamba, Leaf	Its bark is used for urinary infections and biliousness. It is used for diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds and ulcers.
3	*Argyreia speciosa* L.f.	Convolvulaceae	Brudhadaraka,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 for 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 for 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 infection. 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 cystitis, gastritis, enteritis and diarrhoea.
11	Cissus quadrangularis L.	Vitaceae	Hadajoda, Leaf	It is useful in eye and ear diseases and colic, leprosy, ulcers, tumours 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*	Asteraceae	Mayurachulia, Leaf	Roots and leaves are reported for diarrhoea, dysentery, swellings and stomach pain. Powdered with pepper it is applied for toothache. Leaves are used in applications for eczema and ulcers.
14	*Ficus glomerata* Roxb	Moraceae	Dumer, Leaf	Leaf decoction are used against dysentery, diabetes, stomachache, piles and diarrhoea.
15	*Glycyrrhiza glabra* L.	Fabaceae	Yasthimadhu, Leaf	It is useful in cough, bronchitis, ulcer, fever, hoarseness of voice, skin diseases, eye diseases, 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 is applied to 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 a potent antitubercular remedy and used to cure liver and is useful in diarrhoea. It is also used in fever, inflammations, amenorrhoea, dysmenorrhoea, 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	Raktachandan Leaf/ Bark	It is used as an antiseptic, wound healing agent and anti-acne treatment. A decoction of fruit is used for chronic dysentery.
20	*Salvadora persica* Wall	Salvadoraceae	Meswak, Bark	Leaves are useful in asthma, bronchitis, cough, painful tumors and verminosis. Shoots and leaves are used in treatment of cough and bronchitis. Tender twigs are used as toothbrush.
21	*Tectona grandis* L.	Lamiaceae	Teak, Bark	It is used as an antiseptic, wound healing agent and antiacne treatment
22	*Terminalia alata* Heyene ex Roth	Combretaceae	Sahaj, Leaf	For epilepsy, diarrhoea, dysentery aliquots of 20-30 ml of bark paste 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 an 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 a 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 enteropathogenic bacteria from clinical samples (stool) and their colony characteristics.

Bacterium	Media	Colony characters
Enterobacter aerogenes	Blood agar	White convex with gamma hemolysis
	MC Agar	LF, mucoid
Escherichia coli	Nutrient agar	Flat dry, irregular
	MC agar	LF, flat dry pink, irregular
	EMB agar	Purple coloured, flat dry, irregular colonies, with metallic green colour
Klebsiella sp.	MC agar	LF, pink, mucoid
	CLED Agar	Yellow mucoid
Salmonella paratyphi	MC agar	NLF, colourless
	XLD agar	Red colour, pinpoint colonies with black center
Salmonella typhi	MC agar	NLF, colourless
	XLD agar	Red colour, pinpoint colonies with black center
Shigella boydii	MC agar	NLF, circular, smooth, translucent
Shigella dysenteriae	MC agar	NLF circular, smooth, translucent
Shigella flexneri	MC agar	NLF circular, smooth, translucent
Shigella sonnei	MC agar	LLF, flat with jagged end
Vibrio cholerae	TCBS agar	Smooth, opaque, yellow colour

Note: LF, lactose fermenting; NLF, non lactose fermenting; LLF, late lactose fermenting; CLED, cysteine lactose electrolyte deficient; EMB, eosin methylene blue; MC, MacConkey; TCBS, thiosulfate-citrate-bile salts-sucrose; XLD, xylose lysine deoxycholate

Table 3.

Biochemical identifications of the isolated MDR enteropathogenic bacteria

Bacterium (MDR strain)	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate
E. aerogenes	+	−	−	−	+	+	−	A/A	+
E. coli	+	−	+	+	−	−	−	A/AG	+
Klebsiella sp.	+	−	−	−	+	+	+	A/AG	+
S. paratyphi	+	−	−	+	−	+	−	K/A	+
S. typhi	+	−	−	+	−	+	−	K/A /H2S	+
S. boydii	+	−	−	+	−	−	−	K/A	+
S. dysenteriae	+	−	+	+	−	−	−	K/A	+
S. flexneri	+	−	+	+	−	−	−	K/A	+
S. sonnei	+	−	−	+	−	−	−	K/A	+
V. cholerae	+	+	+	−	−	+	−	nd	+

Note: 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; +, positive; −, negative. Abbreviations: MR, methyl red; VP, Voges-Proskauer; TSI, triple-sugar-iron.

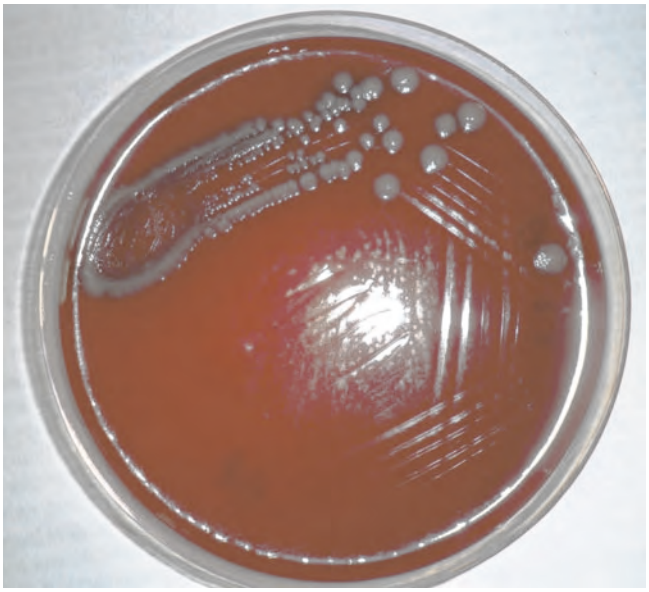

Fig 1. Colonies of *Enterobacter aerogenes* showing white convex with gamma hemolysis on blood agar

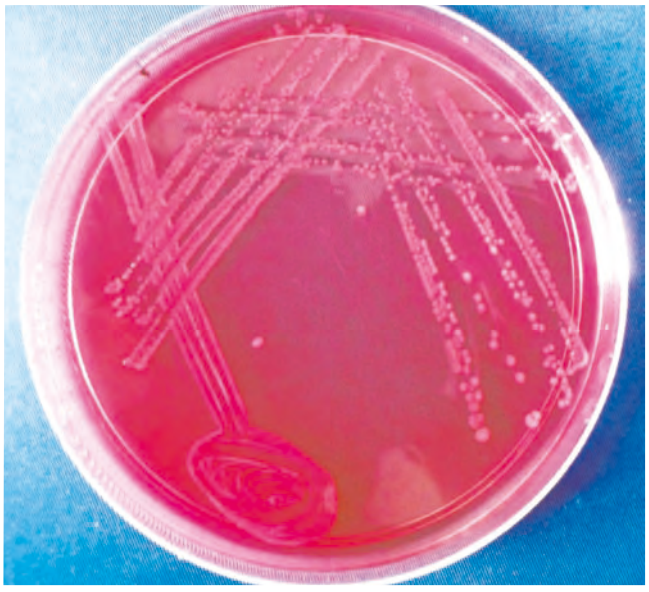

Fig 2. Lactose fermenting colonies of *Escherichia coli* colonies on MacConkey agar

One strain of each of 10 enteropathogens was further selected for antibiotic profiling and for monitoring antibacterial activities of all cited plants. *E. coli* (Figure 2) was found sensitive to ciprofloxacin, co-trimoxazole and chloramphenicol whereas, that was found resistant to amikacin, amoxyclav, ampicillin, ceftriaxone, cefpodoxime, gentamicin, gatifloxacin, nalidixic acid, nitrofurantoin, norfloxacin, ofloxacin, piperacillin/tazobactam and tetracycline, at specified levels of each antibiotic. Similarly, antibiotic sensitivity patterns of 9 other pathogens, using one strain of each, were recorded (Table 4). It was discernible that strains of *Klebsiella* sp. and *S. sonnei* were resistant to 14 out of 16 antibiotics, and *V. cholerae* had resistance for 4 out of 16 antibiotics used.

A total of 45 (11.8%) and 14 (5.14%) isolates of *E. aerogenes* were isolated from HA and CA samples, respectively; similarly, details of numbers and percent values of the rest 9 bacteria are presented in Table 5. In both HA (380=100%) and CA (272=100%) clinical samples, *E. coli* isolates were of the highest numbers, 173 (45.5%) and 167 (61.39%), respectively. *Klebsiella* sp. was the second leading organism isolated in both HA and CA samples with numbers 145 (38.16%) and 79 (29.04%), respectively. Further, percent values of each of 10 pathogens resistant to individual drugs of 6 groups of antibiotics are also presented in Table 6. For example, *E. coli* had the highest 93% resistance among HA isolated strains, while 89% resistance among CA isolates to gentamicin 10 μg/disc.

While monitoring the antibacterial properties of 25 plants, it was evident that both aqueous and ethanolic extracts of plants, *Aegle marmelos*, *Azadirachta indica*, *Cassia*

Table 4.
Antibiotic susceptibility results of the selected clinically isolated enteropathogenic organisms.

Bacterium	Susceptibility to prescribed antibiotics															
	Aminoglycosides		β -lactams			Cephalosporins		Fluoroquinolones					Sulfonamide	Stand alones		
	Ac	Ge	Am	Ak	Pt	Ce	Cf	Ci	Gf	Na	No	Of	Cot	Ch	Nf	Te
E. aerogenes	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	I
E. coli	R	R	R	R	R	R	R	S	R	R	R	R	S	S	R	R
Klebsiella sp.	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R	R
S. paratyphi	S	S	R	R	S	R	R	S	R	S	S	S	R	S	I	S
S. typhi	R	R	R	R	R	R	R	I	R	R	R	R	R	S	R	R
S. boydii	R	R	I	R	R	R	R	S	S	R	R	S	S	S	R	S
S. dysenteriae	R	I	R	R	R	R	R	S	R	R	R	R	R	S	R	R
S. flexneri	R	R	R	R	I	R	R	R	R	R	I	I	S	S	R	I
S. sonnei	R	R	R	R	R	R	R	S	R	R	R	R	R	S	R	I
V. cholerae	R	R	S	S	S	R	S	S	S	S	S	S	R	S	S	S

Note: 'R'- Resistant; 'S'- Sensitive; 'I'- moderately sensitive; 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: norfloxacin 10; Of: ofloxacin 5; Pt: piperacillin/tazobactam 100/10; Te: tetracycline 30.

fistula, *Holarrhena antidysenterica*, *Salvadora persica* and *Terminalia arjuna* were highly effective against all the isolated enteropathogens; plants, *Ficus glomerata*, and *Pterocarpus santalinus* had limited activity. Both aqueous and ethanolic extracts of *Glycyrrhiza glabra* showed a moderate antibacterial activity against *S. boydii*, *Klebsiella* sp. and *S. flexneri* (Tables 7). The categorization of highly and moderately effective plant extracts are detailed (Table 8). In general, the ethanolic extracts had better/ significant antibacterial activity than the corresponding aqueous extracts.

Table 5.

Hospital acquired and community acquired accounts of enteropathogens (total n= 380+272=652) in a span of 6 months.

Bacterium	Number of isolates	
	HA (%)	CA (%)
E. aerogenes	45 (11.8)	14(5.14)
E. coli	173(45.5)	167(61.39)
Klebsiella sp.	145(38.16)	79(29.04)
S. paratyphi	2(0.52)	1(0.36)
S. typhi	4(1.05)	3(1.10)
S. boydii	1(0.26)	1(0.36)
S .dysenteriae	5(1.31)	3(1.10)
S. flexneri	1(0.26)	−(−)
S. sonnei	3(0.78)	2(0.73)
V. cholerae	1(0.26)	2(0.73)
Total	380 (100)	272(100)

Note: HA, hospital acquired; CA, community acquired. Numbers in parenthesis are percentages of occurrence.

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. Presence of such phytocompounds in individual extracts cumulatively redounds to the antibacterial activities of plants. The results of phytochemical analysis of all plants were recorded (Table 9). All these used plants had phytochemicals in aqueous and ethanolic extracts in the following profusion out of 25 plants: aqueous (ethanolic), alkaloids 16(21), glycosides 17(20), terpenoids 15(24), reducing sugars 19(21), saponins 17(22), tannins 19(24), flavonoids 22(24) and steroids 16(22). The aqueous but not ethanolic extract of *C. fistula* contains terpenoids, for example.

Figure 3. *Cassia fistula* (Indian laburnum).

Table 6.

Antibiotic resistance pattern of the isolated enteropathogens in the span of 6 months

Strains	Percent values of resistant isolates to individual antibiotics															
	Aminoglycosides		β −lactams			Cephalosporins			Fluoroquinolones				Sulfonamide	Stand alones		
	Ak	Ge	Am	Ac	Pt	Ce	Cf	Ci	Gf	Na	No	Of	Cot	Ch	Nf	Te
Enterobacter aerogenes	56 (34)	65(83)	75(62)	54(34)	61(45)	68(35)	45(12)	57(47)	53(37)	34(26)	56(23)	51(36)	39(19)	24(21)	78(67)	38(24)
Escherichia coli	78(71)	93(89)	61(57)	79(51)	71(68)	49(45)	89(78)	91(84)	86(75)	67(63)	78(54)	67(39)	83(71)	56(35)	89(83)	59(28)
Klebsiella sp.	87 (76)	83(57)	89(78)	59(53)	45(20)	76(65)	81(75)	76(67)	94(91)	59(47)	83(76)	63(56)	71(61)	45(34)	75(48)	68(63)
Salmonella paratyphi	50(−)	100(100)	−(−)	−(−)	−(−)	−(−)	−(100)	−(−)	50(100)	100(−)	−(−)	−(−)	50(100)	−(−)	−(−)	−(−)
S. typhi	50(66)	75(34)	25(34)	25(66)	50(−)	25(−)	100(100)	75(100)	25(66)	50(35)	50(66)	−(34)	25(100)	−(−)	50(100)	−(−)
Shigella boydii	100(100)	100(100)	100(100)	100(100)	−(−)	100(−)	100(−)	100(−)	−(100)	100(100)	100(100)	−(−)	−(−)	−(−)	100(100)	−(−)
S. dysenteriae	40(66)	100(66)	60(34)	20(−)	20(−)	80(34)	−(66)	60(34)	100(66)	40(66)	60(66)	−(34)	40(100)	60(−)	60(100)	−(−)
S. flexneri	100(−)	100(−)	100(−)	100(−)	−(−)	100(−)	−(−)	−(−)	100(−)	100(−)	100(−)	−(−)	100(−)	−(−)	100(−)	−(−)
S. sonnei	34(−)	100(100)	34(50)	66(−)	34(50)	66(−)	−(50)	34(34)	100(100)	34(50)	66(50)	34(−)	34(100)	−(−)	66(100)	−(50)
Vibrio cholerae	−(50)	100(100)	100(50)	−(−)	−(−)	−(−)	−(50)	−(−)	100(50)	100(−)	−(50)	−(−)	100(50)	−(−)	−(50)	−(−)

Note: Antibiotics (μ g/disc): For abbreviation of antibiotics and their levels, see Table 4. Numbers denote from HA isolates (n=380) and numbers in parenthesis "( )" denote from CA isolates (*n*=272).

Table 7a.
Result of screening of selected medicinal plants by the agar cup method.

Bacteria	Zone of inhibition by thirteen plant extracts (mm)												
	1	2	3	4	5	6	7	8	9	10	11	12	13
E. aerogenes	13 (16)	_	_	19(23)	15(17)	17(20)	15 (18)	11(14)	15(19)	13 (15)	12(15)	17(19)	7(20)
E. coli	19(22)	7 (13)	16(19)	19(25)	21(23)	19(21)	13 (16)	16(19)	22(25)	14(15)	13(15)	21(23)	18(21)
Klebsiella sp.	17(23)	13 (15)	18 (19)	18(22)	17(21)	16(18)	_	_	18(20)	11(16)	16(18)	_	6(18)
S. paratyphi	16(18)	_	_	15(18)	_	_	15(19)	_	18(21)	_	14(18)	8(11)	9(21)
S. typhi	19(21)	_	9(13)	16(18)	16(18)	_	14(18)	11(16)	15(18)	_	11(15)	11(14)	_
S. boydii	11(12)	_	_	21(23)	20(24)	_	_	_	21(24)	_	_	_	11(13)
S. dysenteriae	17(19)	_	12(17)	11(13)	12(13)	_	21(23)	16(17)	21(23)	13(15)	_	_	21(23)
S. flexneri	16(17)	_	_	9(11)	_	_	19(21)	_	15(17)	12(15)	_	_	
S. sonnei	18(21)	_	_	12(15)	12(16)	_	22(24)	_	17(19)	17(19)	_	_	_
V. cholerae	9(13)	_	6 (10)	12(13)	14(17)	_	13 (16)	_	14(17)	_	_	_	_

Note: Numbers 1 to 13 are serial numbers of plants given in Table 1; Values outside parenthesis are measurements of zone of inhibition due to aqueous extracts and values in parenthesis are due to ethanolic extracts. "_" sign denotes no activity.

Table 7b.
Result of screening of selected medicinal plants by the agar cup method.

Bacteria	Zone of inhibition by twelve plant extracts (mm)											
	14	15	16	17	18	19	20	21	22	23	24	25
E. aerogenes	_	_	23(26)	14 (17)	_	_	15 (18)	13 (17)	17(19)	21(23)	16(19)	_
E. coli	14(16)	_	22(26)	19(22)	22(25)	_	13 (16)	18 (21)	21(23)	25(26)	18(21)	24(26)
Klebsiella sp.	_	9(13)	17(21)	19(23)	_	15(17)	_	14(17)	15(18)	17(19)	22(24)	_
S. paratyphi	_	_	15(17)	14(16)	18(21)	_	17(19)	16(19)	14(11)	18(20)	_	18(19)
S. typhi	_	_	15(18)	19(21)	15(18)	_	16(19)	21(27)	12(13)	15(18)	_	15(18)
S. boydii	_	18 (19)	21(23)	11(12)	21(24)	13(15)	_	12(14)	16(12)	18(21)	_	_
S. dysenteriae	12(15)	_	21(23)	17(20)	21(23)	_	21(24)	21(24)	14(17)	20(23)	21(23)	18(22)
S. flexneri	_	6 (10)	20(22)	16(17)	15(17)	_	18(21)	17(22)	12(15)	20(24)	22(26)	16(18)
S. sonnei	_	_	19(23)	18(21)	17(19)	_	22(24)	21(25)	15(17)	19(23)	17(21)	19(21)
V. cholerae	_	_	12(15)	9(11)	_	_	13 (16)	_	_	16(18)	15(17)	14(15)

Note: Numbers 14 to 25 are serial numbers of plants given in Table 1. Also see Table 7a for details of zone of inhibition.

4. Discussion

From antibiograms, it could be concluded that these pathogens were adequately MDR, signifying their subtle infection dynamics. For example, *E. aerogenes* was resistant to 14 antibiotics excluding ciprofloxacin and chloramphenicol. Similar situations were seen with other pathogens, which clearly indicated the chicanery from multidrug resistance. This situation clearly demonstrated the resurgence of well known diseases of the past, i.e., the pre-antibiotic era. Further, enteropathogens are suspected to have zoonotic concerns, as the household animal species and the wild ones are known to be reservoirs of these bacteria, and consequently they contaminate the inland water bodies with their fecal deposits [17]. Eventually, the marginalized people who live in unhygienic conditions continue to be the most affected mass by the enteropathogens. About 2.4 million children continue to die each year because of enteric diseases in unhygienic environment [18]. Furthermore, rapid urbanization has led to overcrowding, poor housing conditions and poor sewage disposal system, which add to infections from all sorts of pathogens to all peoples, but children remain to be the most venerable ones to enteropathogens in developing areas of developing countries. Thus, child morality within the age 5 years has become a commonplace of poverty stricken areas of developing countries, worldwide [10, 14].

Cholera continually has sporadic outbreaks in many parts of India [6], because of its development of serological subtypes, and one virulent strain was reported from New Delhi [19]. A study from Kolkata on *V. cholerae* O1 indicated that its resistance to tetracycline led to the susceptibility of children below 2 years of age; *S. flexneri* and *E. coli* are the causes of morbidity and mortality from diarrhoea, including polymicrobial enteropathogenic infections, in which a rotavirus was often detected [20]. Resistance of cefpodoxime and extended spectrum cephalosporins in *S. typhi* had been described in an Indian study [21]. Reemergence of *S. dysenteriae* was reported from North India with the determinant role of plasmids in conferring resistance to 12 antibiotics in a gap of 10 years of study [22].

Four species of *Shigella* were MDR and of them the most prevalent ones in Nepal were arranged in decreasing order, *S. dysenteriae* (42%), *S. flexneri* (38%), *S. sonnei* (15%) and *S. boydii* (4%), as reported; all these strains were resistant to ampicillin, co-trimoxazole, nalidixic acid

Table 8.
Detailed results of antibacterial activity of leaves of 25 plants obtained from agar well diffusion method.

Sl. no	Plants	Aqueous extract		Ethanolic extract	
		High susceptibility	Moderate susceptibility	High susceptibility	Moderate susceptibility
1	*A. marmelos*	–	All isolates, **V. cholerae* **S. boydii*	*E. coli*, *Klebsiella* *S. typhi*, *S. sonnei*	*E. aerogenes*, *S. paratyphi* *S. dysenteriae*, *S. flexneri* **V. cholerae*, **S. boydii*
2	*A. cadamba*	–	**E. coli*, *Klebsiella*	–	**E. coli*, *Klebsiella*
3	*A. speciosa*	–	*E. coli*, *Klebsiella*. *S. dysenteriae*,**S. typhi*, **V. cholerae*	–	*E. coli*, *Klebsiella*, *S. dysenteriae*. **S. typhi*,**V. cholerae*
4	*A. indica*	*S. boydii*	*E. aerogenes*, *E. coli*, *Klebsiella*, *S. paratyphi*, *S. typhi*, **S. dysenteriae* **S. flexneri*, **S. sonnei*, **V. cholerae*	*S. boydii*, *E. aerogenes*, *E. coli*, *Klebsiella*	*S. paratyphi*, *S. typhi*S. dysenteriae*, **S. flexneri*, *S. sonnei*,**V. cholerae*
5	*B. monnieri*	*S. boydii*, *E. coli*	*E. aerogenes*, *Klebsiella*, *S. paratyphi*, *S. typhi*, **S. sonnei*,**S. dysenteriae*, *V. cholerae*	*S. boydii*, *E. coli*, *Klebsiella*	*E. aerogenes*, , *S. paratyphi*, *S. typhi*,**S. dysenteriae*, *S. sonnei*, *V. cholerae*
6	*B. monosperma*	–	*E. aerogenes*, *E. coli*, *Klebsiella*	*E. aerogenes*, *E. coli*,	*Klebsiella*
7	*C. procera*	*S. dysenteriae*, *S. sonnei*	*E. aerogenes*, **E. coli* *S. paratyphi*,**S. typhi*, *S. flexneri*,**V. cholerae*	*S. dysenteriae*, *S. sonnei*	*E. aerogenes*, *E. coliS. paratyphi*, *S. typhi*, *S. flexneri*, *V. cholerae*
8	*C. sinensis*	–	**E. aerogenes*, *E. coli*, **S. typhi*, *S. dysenteriae*	–	**E. aerogenes*, *E. coli* *S. typhi*, *S. dysenteriae*
9	*C. fistula*	*S. boydii*, *E. coli*, *S. dysenteriae*	*E. aerogenes*, *Klebsiella*, *S. paratyphi*, *S. typhi*, *S. flexneri*, *S. sonnei* **V. cholerae*	*S. boydii*, *E. coli*, *S. dysenteriae*, *Klebsiella*, *S. paratyphi*	*E. aerogenes*, *S. typhi*, *S. flexneri*, *S. sonnei*, *V. cholerae*
10	*C. roseus*	–	**E. aerogenes*, **Klebsiella*, **E.coli*,**S. flexneri* **S. dysenteriae*, *S. sonnei*	–	*E. aerogenes*, *E. coli*, *Klebsiella*, *S. flexneri*, *S. dysenteriae*, *S. sonnei*
11	*C. quadrangularis*	–	**E. aerogenes*, **E. coli* *Klebsiella*, **S. paratyphi*, *S. typhi*	–	*E. aerogenes*, *E. coli*, *S. typhi*, *Klebsiella*, *S. paratyphi*,
12	*C. collinus*	*E. coli*	*E. aerogenes*, **S. paratyphi*, **S. typhi*	*E. coli*	*E. aerogenes*, **S. paratyphi*, **S. typhi*
13	*E. scaber*	*E. aerogenes*, *S. dysenteriae*	**E. coli*, **S. dysenteriae*	*E. aerogenes*, *S. paratyphi* *S. dysenteriae*, *E. coli*	** S. boydii*, *Klebsiella*,
14	*F. glomerata*	–	**E. coli*, **S. dysenteriae*	–	*E. coli*, *S. dysenteriae*
15	*G. glabra*	–	*S. boydii*, **Klebsiella*, **S. flexneri*	–	*S. boydii*, **Klebsiella*, **S. flexneri*
16	*H. antidysenterica*	*E. aerogenes*, *S. boydii*, *E. coli* *S. dysenteriae*, *S. flexneri*	*Klebsiella*, *S. paratyphi*, *S. typhi*, *S. sonnei*,**V. cholerae*	*E. aerogenes*, *E. coli*, *S. boydii*, *Klebsiella*, *S. flexneri*, *S. sonnei* *S. dysenteriae*	*S. paratyphi*, *S. typhi*, **V. cholerae*
17	*M. oleifera*	_	**E. aerogenes*, *E. coli*, **E. faecalis*, *S. sonnei*, *Klebsiella*,**S. paratyphi*, *S. typhi*, *S. dysenteriae*, *S. flexneri*, **V. cholerae*	*S. typhi*, *S. dysenteriae*, *S. sonnei*, *E. coli*, *Klebsiella*	*E. aerogenes*, **E. faecalis*, *S. paratyphi*, *S. flexneri*, **V. cholerae*
18	*O. indicum*	*E. coli*, *S. boydii*, *S. dysenteriae*	*S. paratyphi*, *S. flexneri*, *S. sonnei*, *S. typhi*	*E. coli*, *S. dysenteriae*, *S. paratyphi*, *S. boydii*	*S. flexneri*, *S. sonnei*, *S. typhi*
19	*P. santalinus*	–	**S. boydii*, *Klebsiella*	–	*S. boydii*, *Klebsiella*
20	*S. persica*	*S. dysenteriae*, *S. sonnei*	*E. aerogenes*, **E. coli*, *S. paratyphi*, *S. typhi*, *S. flexneri*, **V. cholerae*	*S. flexneri*, *S. sonnei* *S. dysenteriae*	*E. aerogenes*, *E. coli*, *S. paratyphi*, *S. typhi*, *V. cholerae*
21	*T. grandis*	*S. typhi* *S. dysenteriae* *S. sonnei*,	**E. aerogenes*, *E. coli*, **E. faecalis*, *Klebsiella*, *S. paratyphi S. flexneri*	*E. coli*, *S. typhi*, *S. dysenteriae*, *S. sonnei*, *S. flexneri*	*E. aerogenes*, **E .faecalis*, *Klebsiella*, *S. paratyphi*
22	*T. alata*	*E. coli*	*E. aerogenes*, **S. typhi*, *S. boydii*S. paratyphi*, *S. flexneri*, *S. sonnei*, **S.dysenteriae*,	*E. coli*	*E. aerogenes*, *S. typhi*, *S. sonnei*, **S. paratyphi*, *S. flexneri*, **S. dysenteriae*,**S. boydii*

23	*T. arjuna*	*E. aerogenes*, *E. coli*, *S. dysenteriae*, *S. flexneri*	*S. paratyphi*, *S. typhi*, *V. cholerae*, *S. sonnei*, *Klebsiella*, *S. boydii*	*E. aerogenes*, *E. coli*, *S. dysenteriae*, *S. sonnei*, *S. flexneri*, *S. paratyphi*, *S. boydii*	*S. typhi*, *Klebsiella*, *V. cholerae*,
24	*W. somnifera*	*Klebsiella*, *S. flexneri*, *S. dysenteriae*	*E. aerogenes*, *E. coli*, *S. sonnei*, *V. cholerae*	*Klebsiella*, *S. flexneri*, *S. dysenteriae*, *E. coli*,	*E. aerogenes*, *V. cholerae*
25	*V. negundo*	*E. coli*	*S. paratyphi*, *S. typhi*, *S. dysenteriae*, *S. flexneri*, *S. sonnei*,*V. cholerae*	*E. coli*, *S. dysenteriae*, *S. sonnei*	*S. paratyphi*, *S. typhi*, *S. flexneri*, *V. cholerae*

Note:* Bacteria were considered showing the least susceptibility to plant extracts had diameters of zones of inhibition less than 10 mm; bacteria were considered showing moderate susceptibility had diameters of zones of inhibition between 10 and 20 mm for both aqueous and alcoholic extracts; and bacteria were considered showing high susceptibility had diameters of zones of inhibition more than 20 mm for both aqueous and alcoholic extracts of plants.

Table 9.

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*	+ (+)	− (+)	+ (+)	+ (−)	− (+)	+ (+)	+ (+)	+ (+)

Note: "+" sign denotes presence, and "−"sign denotes absence of the compound in a plant; sign outside parenthesis denotes about a phytochemical in water extract, and sign in parenthesis denotes in ethanolic extract.

and second line drugs, ciprofloxacin and mecillinam [23]. From Singapore, many MDR Enterobacteriaceae members (*Citrobacter*, *Enterobacter*, *Klebsiella* sp. and a few more) were resistant to ampicillin, third generation cephalosporins and aminoglycosides [24]. A Canadian work demonstrated drug resistance against *Salmonella*, *E. coli* and *Shigella* [25]. A Brazilian work recorded the prevalence of *E. coli* and *Enterobacter cloacae* with MDR genotypes and efflux systems with proton motive force evading 12 antibiotics of different classes including cefaclor and spiramycin [26]. The mechanism of resistance of *E. coli* and *Klebsiella* to levofloxacin, ciprofloxacin and prulifloxacin was reported from Italy with the involvement of mutation of gyrase–encoding sequences of gyrA, gyrB, parC and parE genes [27]. In a surveillance system in 23 European countries, the highest rate of resistance was recorded for *K. pneumoniae* with 46 % resistance to carbapenems, 58% to quinolones, and 63% to cephalosporins, and the resistance mechanism was linked to regulated efflux pumps of pathogens *E. coli*, *Enterobacter* sp. and *Serratia* sp. [28, 29]. Shigellosis in Iran was found to be due to MDR strains resistant to streptomycin, co–trimoxazole, tetracycline, ampicillin, nalidixic acid and kanamycin, but was found to be sensitive to ceftriaxone, ceftazidime, cephalothin and cefotaxime. Drug resistant strains of *S. sonnei* were seen to have class II integrons with 137 base pairs [30].

Another study on enteric bacteria with *E. coli* and *Shigella* sp. indicated crude extracts of plants, *Hemidesmus indicus*, *Holarrhena antidysenterica* and *Plumbago zeylanica* recorded high control ability on MDR ESBL *E. coli* and *Shigella* [31]. Plants, *Terminalia chebula* and *Syzygium cumini* had been recorded for broad spectrum antibacterial activity against *V. cholerae*, *Aeromonas hydrophila*, especially for cholera and diarrhoea causing bacteria with minimum bactericidal concentrations (MBCs), ranging from 0.25 to 4.0 mg/mL [32]. Of 25 plants used herein, only *A. marmelos*, *B. monnieri*, *C. sinensis*, *C. quadrangularis* and *M. oleifera* did 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 [33], 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 10 isolated MDR bacteria. Further, the iconic plant of India, *A. indica* has been reported to have the control over 33 strains of many organisms including *Klebsiella* and *Enterobacter* [34].

In a study, *Cassia fistula* (Indian laburnum) seeds had a significant control over *E. coli* and *S. typhi* and the minimum inhibitory concentration (MIC) values were found in the range of 1.563 to 50.00 mg/mL [35]. *Terminalia arjuna* showed promising *in vitro* control over *S. typhi*, *S. paratyphi*, *V. parahaemolyticus*, *V. minus*, *E. coli*, *S. boydii* and *S. dysenteriae* [36]. Indeed, plants have antimicrobial properties due to 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 the rise, 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 [37].

Antibiotics have been the sum and substance of clinical management today. But, distressingly resistance of antibiotics by the pathogenic bacteria particularly of *S. aureus*, *E. coli*, *P. aeruginosa*, *Acinetobacter*, *Klebsiella*, *Enterococcus*, *Proteus* and a few more have become a commonplace and the intractable MDR *S. aureus* has become the superbug in the health domain. With simple genomes, bacteria have intra-specific gene transfer mechanisms operative in nature normally [38]. But the camaraderie of microbes have helped even inter-generic gene transfers so that, as an epitome, the 'multiple antibiotic resistant' locus (*mar* locus) of *E. coli* has been reported even to work in the most phylogenetically distant *Mycobacterium smegmatis* [38], raising a high level of clinical consternation all over. Not surprisingly, an antibiotic never applied for a pathogen is found as resistant to a gamut of pathogens, in many instances[39].

In conclusion, paradigmatically herein, all strains of isolated enteropathogens including the life-threatening *V. cholerae* were MDR. Enteropathogens, particularly contaminating the drinking water system and inland water bodies of countries like India and many, continue to cause substantial number of child mortality. So, it would be a medical infraction, if due steps are not initiated against this class of MDR pathogens. This work signifies that phytochemicals have good antimicrobial activity *in vitro* on the cohort of notorious MDR enteropathogens as non-microbial antimicrobials. Thus, phytochemicals can be accentuated as complementary medicines and scaling up of their use as antimicrobials could help the pharmacy world.

Conflict of interest statement

We declare that we have no conflict of interest.

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