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Research Article

In vitro Efficacy of 30 Ethnomedicinal Plants Against Clinically Isolated Gram-Negative MDR Bacteria

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Abstract

Objectives: Ethanol and aqueous leaf extracts of 30 common and non-common plants used by aborigines of Kalahandi district, Odisha, were used for antibacterial activities in vitro against Gram-negative clinically isolated bacteria were of 11 genera, A*cinetobacter, Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Pseudomonas, Salmonella, Serratia, and Shigella*.

Materials and Methods: The antibiotic sensitivity patterns of all bacterial strains were studied with the disk-diffusion method with 17 antibiotics belonging to 8 classes by Kirby–Bauer method. All isolated bacteria were amply multidrug resistant (MDR). Monitored plants have ethno-medicinal uses and several are used as traditional medicines. Antibacterial properties were studied with the agar-well diffusion method. Minimum inhibitory concentration and minimum bactericidal concentration values of ethanolic and aqueous extracts of plants were determined by microbroth-dilution method.

Results: Ethanolic plant-extracts had the better antibacterial potencies in comparison to their corresponding aqueous extracts. Plants with most conspicuous antibacterial properties in controlling MDR strains of Gram-negative bacteria were aqueous and ethanolic extracts of plants, *Carthamus tinctorius, Cucurbita maxima, Murraya koenigii, Leucas aspera, Plumbago indica* and *Psidium guajava*. Ethanolic extracts of most plants had phytochemicals, alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids.

Conclusion: These plants could be used further for the isolation of pure compounds to be used as complementary non-microbial antimicrobial medicines.

Keywords: Ethnobotany; Antibacterial Property; MDR Bacteria; Phytochemical Analyses; Minimum Bactericidal Concentration

Introduction

The poverty-stricken and marginalized section in India consisting of aborigine tribes living in hilly areas continue to depend on plant/herbal products from the local forest-patch for all basic needs including the health care. Plants, for the health-care needs of the numerically important aborigine tribe, Kandha tribe, were described from Odisha state [1,2]. Located at the eastern range of mountains in the state, with a 40% aborigine population, Kalahandi district is richer in vegetations in comparison to other hilly patches of the state. These people maintain their ethnomedicinal knowledge orally in a surreptitious way down the generations, but young adults of the society migrate from their base for livelihood to urban areas; eventually, they lose the attention from medicinal plants. Moreover, regular episodes of summer forest fire in the district as in several other places of Indian forest patches lead to inexorable and insurmountable loss of vegetations, entailing environmental degradations at all angles of fire catching zones [3]. Furthermore, as if adding fuel to fire, unsustainable harnessing of prodigious forest products including plant parts for the medicinal plant trade and timber causes a blasting diminution of phytodiversity and concomitant shape-shifting of the total forest that becomes unsuitable to contain the usual flora and fauna. Eventually, the creation of forest patches becomes too common that induces survey work of medicinal plants at different areas. Furthermore, India with tropical and sub-tropical forest areas is a home to 550 million plants approximately that serve as the source of traditional medicine (TM), derived from the clandestine ethnic information and Ayurveda [4]. Plants involved in TM have been in use in several

ways and they are popular with a'la carte menu-like concoctions and specific modalities, idiosyncratic to 'medicines and diseases', which have facilitated the modern drug development and the use of finished herbal medicines as different formulations, in the 'Herbal-medicine-trade' [5]. TM as a field remains as the major accessible and affordable method of treatment for health of marginalized people and aborigines, as the old social paradigm. Moreover, TM has been in use in several developed western countries, as an important mode of complementary and alternative medicine (CAM) (Altman, 2008; Lee and Bielory, 2010; Pineda and Singh, 2012) [6-8]. For example, a 48% population in Australia, a 70% in Canada, a 42% in USA, a 38% in Belgium, and a 75% in France use CAM, as it is known [9]; nevertheless, the most popular herbal medicines of the trade do not have institutional/scientific/clinical/pharmaceutical validation for the direct use as drugs in the mainstream medicine. Such crude phyto-drugs are available in market shelves everywhere and elite people love to lean to them, for well-being or health boosting. Many a crude concoctions of phyto-drugs are preventives, but their curative roles are mostly not established.

The aim of this work was to verify 30 common and non-common plants used by aborigines of Odisha, in an attempt to identify their control over all clinically isolated Gram negative (GN) pathogenic bacterial strains *in vitro*. Antibiograms of those isolated bacteria with 17 antibiotics of the day ascertained that could help landing at the conclusion that all were amply MDR. Thus, work on individual plants in controlling MDR strains of bacteria was recorded. Values of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) with phyto-extracts of MDR bacteria had been recorded. All these plants have ethnomedicinal uses and many of them are used as TM. The recorded data are anticipated to trigger work on the isolation of pure compounds for further scientific use in the crusade of the control of MDR pathogens. This is a record of scientific verification of ethnomedicinal information on a group of plants from Kalahandi forest, in continuation to the previous work2, and for the possible use of these plants as CAM.

Materials and Methods

- **Survey work:** Plants reported (listed in Table 1, Figure 1a-f, Figure 2a-f) were collected from Kalahandi forest during December 2011; 15 hamlets (villages) of Junagarh block of Kalahandi district were surveyed. Junagarh is situated at 19°.10' and 20°.30' north latitude and 82°.30' and 83°.50' east longitude; other details of the district were stated elsewhere [2].
- **Preparation of plant extracts:** A lot of 20 g of powder from clean leaf-samples was dissolved separately in aliquots of 200 mL sterile double-distilled water and 200 mL 80% ethanol, in wide-mouth bottles and bottles were incubated at room temperature for 48 h. Details of extraction were stated [5]; 0.02 to 1.4 g solid sticky mass/20 g leaf-powders of each plant was obtained, dissolved in 2 mL aliquots of 10% dimethyl sulfoxide (DMSO) and was stored at 4°C until further use.
- **Phytochemical analysis of plants:** Plant extracts using ethanol and water were subjected to several chemical tests to know the presence of flavonoids, saponins, phlobatannis, resins, sterols, lipids/fats, steroids, tannins, glycosides, acidic compounds, terpenoids, reducing sugar, phenols, carbohydrates and anthra-quinones [9-11].
- **Isolation of bacteria from clinical samples:** Nutrient broth (NB) and nutrient agar (NA) (HiMedia, Mumbai) were used for bacterial growth. The bacterium intended for Gram-staining was in the log phase of growth. The test bacterial strains isolated and used were the 17 GN bacteria belonging to 11 genera (see Table 3, Figure 3a-f, Figure 4a-f and Figure 5a). These bacterial strains along with MTCC strains as standard reference strains were isolated to pure (axenic) cultures before performing biochemical characterization, accordingly [12].
- **Biochemical identifications and antibiotic sensitivity tests:** For pure-cultures of GN bacilli, biochemical tests were done in succession, as detailed previously [13]. Sugar tests of glucose, lactose, sucrose, maltose and mannitol fermentation were carried out [12]. All the used bacterial strains were subjected to antibiotic sensitivity test by the disc-diffusion/Kirby-Bauer's method (Figure 5b-f), with 17 high potency antibiotic-discs (HiMedia), according to CLSI guidelines [13].
- **Antibacterial activity test by agar-well diffusion method:** On a bacterial lawn wells were punched for 6 mm deep in 30 min old bacterial lawn and each well was based by 50 µL molten Muller-Hilton (MH) agar. 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% v/v, DMSO to 30 mg plant-extract/mL, and that of the aqueous plant-extract with water). Plates were incubated at 37°C for 24 h**.** Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition [13,14]. 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. Sterile water was taken as the control for experiments with both cold aqueous phyto-extracts.
- **MIC and MBC values of plant extracts against isolated bacteria**: Original stock solutions of plant extracts prepared with water and ethanol (cold extracts) were 50 mg/mL in 10% DMSO solution with distilled water. An aliquot of 80 µL of each dilution of a solvent-extract was released to a well on a 96-welled (12×8) micro-titer plate along with an aliquot of 100 µL nutrient NB (HiMedia), an aliquot of 20 µL bacterial inocula (10^9 CFU/mL) and a 5 µL-aliquot of 0.5 % of 2,3,5-triphenyltetrazolium chloride (TTC). After pouring all the above to a well, the micro-plate was incubated at 37°C for 18 h. A pink colouration in a well indicated bacterial growth due to TTC and the absence of any colour was taken as the inhibition of bacterial growth. The first well of the micro-titre plate was the control without any plant extract. The MIC value was noted at the well, where no colour was manifested. Further, bacteria from each well of the micro-plate were sub-cultured on a nutrient agar plate; the level of dilution, where no bacterial growth on the agar plate was observed, was noted as the MBC value [15]. Experiment of each solvent extract was conducted thrice and results of the third repetition are presented.

Figure 1: Plants.

Figure 1a: *Carica papaya*.

Figure 1b: *Musa sapientum*.

Figure 1c: *Psidium guajava*.

Figure 1d: *Diospyros melanoxylon*.

Figure 1e: *Murraya koenigii*.

Figure 1f: *Cucurbita maxima.*

Figure 2: Plants.

Figure 2a: *Azadirachta indica*.

Figure 2b: *Hibiscus rosa-sinensis*.

Figure 2c: *Syzygium cumini*.

Figure 2d: *Thevetia neriifolia*.

Figure 2e: *Punica granatum*.

Figure 2f: *Calotropis procera*.

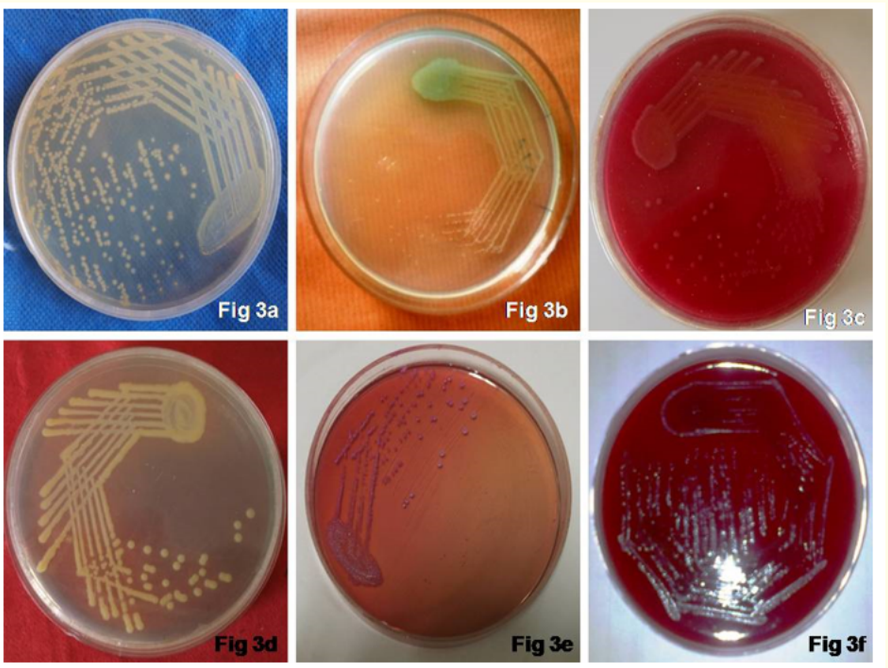

Figure 3: Gram negative Bacterial on different media.

Figure 3a: *Acinetobacter sp. on Nutrient agar.*

Figure 3b: *Pseudomonas aeruginosa on Nutrient agar.*

Figure 3c: *Enterobacter sp. on Macconkey agar.*

Figure 3d: *Citrobacter sp. on Nutrient agar.*

Figure 3e: *Escherichia coli on Macconkey agar.*

Figure 3f: *Shigella flexneri on Macconkey agar.*

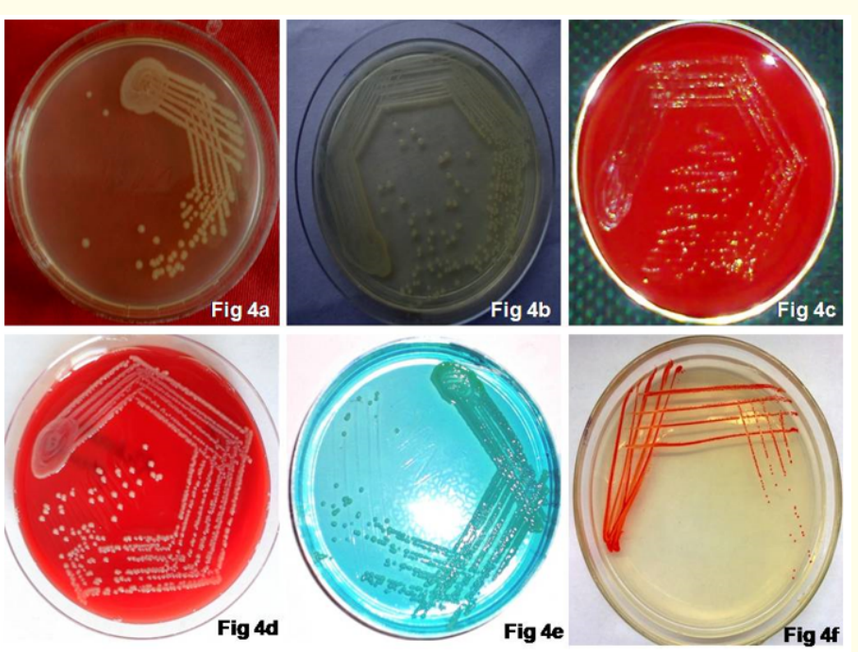

Figure 4: Gram negative bacteria on different media.

Figure 4a: *Salmonella sp. on Nutrient agar.*

Figure 4b: *Salmonella paratyphi A on Nutrient agar.*

Figure 4c: *Shigella dysenteriae on Blood agar.*

Figure 4d: *Klebsiella sp. on blood agar.*

Figure 4e: *Escherichia coli on CLED agar.*

Figure 4f: *Serratia marcescens on Nutrient agar.*

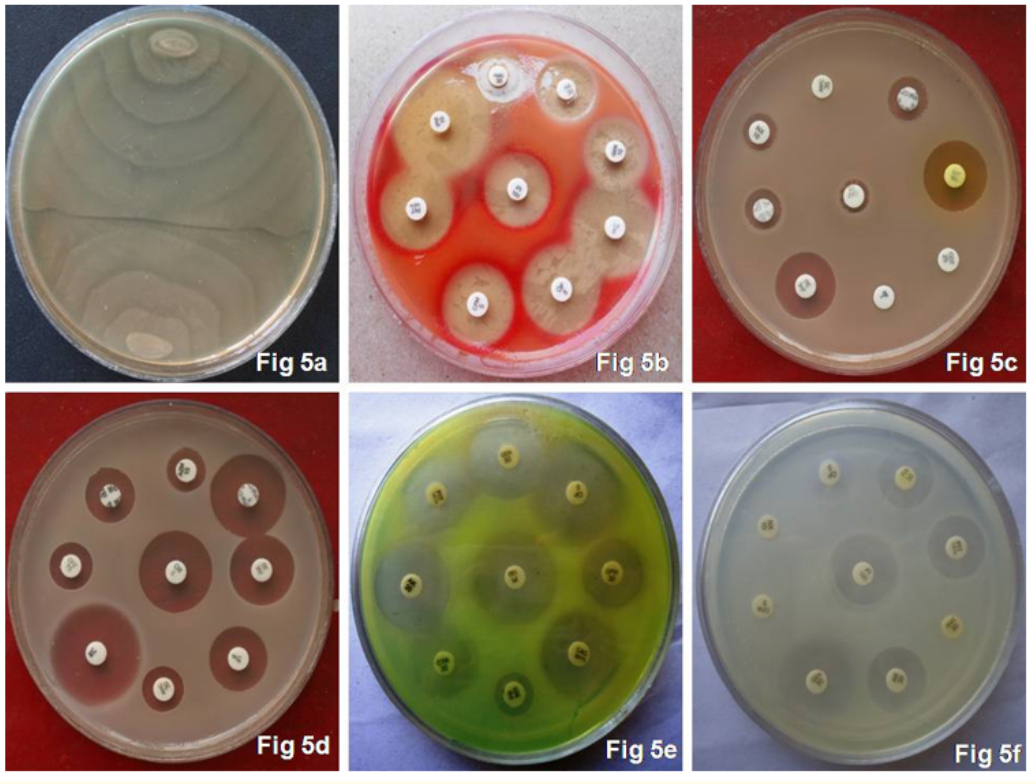

Figure 5: Swarming of *Proteus* sp. and ABST of Gram negative bacteria on MHA.

Figure 5a: Swarming of *Proteus* sp. on MHA.

Figure 5b. ABST pattern of *Serratia marcescens* MHA.

Figure 5c: ABST of *E. coli* on MHA.

Figure 5d: ABST of *K. pneumonia* on MHA.

Figure 5e: ABST of *P. aeruginosa* on MHA.

Figure 5f: *Enterobacter* sp on MHA.

Results

Ethnobotany and preliminary phytochemical analyses: Ethnomedicinal information of 30 plants (of which, 15 were edible) with vernacular names from aborigines of Kalahandi district, Odisha along with their modalities, sometimes with several other plants in use are recorded. These plants are in use for diseases, measles, chicken pox, stomach pain, jaundice, diarrhoea, gonorrhoea, cough, ulcers, skin diseases, inflammations and arthritis, etc. (Table 1). Preliminary phytochemical analyses were done for both aqueous and ethanolic extracts of all plants. Ethanolic extracts of most plants had phytochemicals, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids. However, aqueous extract of certain plants did not contain flavonoids, but corresponding alcoholic extracts had flavonoids. Obviously, the presence of such phytocompounds in individual extracts cumulatively redounds to the antibacterial activities of plants. The results of phytochemical analyses of all plants are recorded (Table 2).

Bacterial identifications: The GN bacterium, Acinetobacter sp. was identified basing on its colony characteristics on NA, and MacConkey (MC) agar as well as from the results obtained from the adopted biochemical procedures: it grew as colourless, smooth, opaque, raised and pinpoint colonies on NA, but as colourless, smooth, opaque, raised and non-lactose-fermenting (NLF) colonies on MC agar. It was found positive to catalase, Voges-Proskauer (VP), citrate and motility tests, whereas negative to oxidase, indole, methyl red (MR) and nitrate tests (Tables 3, 4 and 5). Similarly, all other GN bacteria were identified from biochemical characteristics, as detailed previously [15].

Sl. no	Plant and family name	Local name, (edibility, E or non-edibility, NE)	Ethnomedicinal uses
1	*Amorphophalus campanulatus* Decne. Araceae	Olua (E)	The underground stem is edible and is used for curing stomach pain, treating piles and hemorrhages.
2	*Azadirachta indica A.Juss*. Meliaceae	Nimba (E)	Leaf paste made with *Curcuma longa* (turmeric) is used against measles and chicken pox. It is taken orally as well as applied locally.
3	*Calotropis procera* (Ait.) R. Br. Asclepiadaceae	Arakha (NE)	Leaf juice is dropped in to the nostril to treat epilepsy. Root bark paste with opium is applied externally on nostrils to cure nasal sore.
4	*Cana indica* L. Var Cannaceae	Kedar (NE)	Leaves are used in the treatment of acute jaundice.
5	*Carica papaya* L. Caricaceae	Amrutabhanda (E)	1. Green Fruits of papaya are used to treat high blood pressure, dyspepsia, constipation
6	*Carthamus tinctorius* L Asteraceae.	Kusuma (E)	Leaves are used for amenorrhoea, dysmenorrhoea and wounds or sores with pain and swelling, and for prevention of atherosclerosis.
7	*Cedrus deodara* (Roxb) Loud Pinaceae	Deodar (NE)	Leaf extract is used for catarrhal conditions of the respiratory tract.
8	*Codiaeum variegaum* (L.) Juss Euphorbiaceae	Croton (NE)	Used to treat amenorrhoea, body aches and eye diseases.
9	*Combretum decandrum* Roxb. Combretaceae	Atundi (NE)	The seed oil of the plant is used in treating eczema. The raw leaves are eaten to relieve diarrhoea and gastric troubles.
10	*Cucurbita maxima* Duch.ex Lam. Cucurbitaceae	Kakharu (E)	Fruits are used in treating bladder disorders, stomach upsets, wounds, and certain female reproductive complaints.
11	*Diospyros melanoxylon* Roxb. Ebenaceae	Kendu (E)	Leaves are used in urinary tract infection and skin trouble. Bark is used in diarrhea and dyspepsia.
12	*Euphorbia caducifolia* Haines Euphorbiaceae	Khira siju (NE)	Plants are used for the detection of diabetes mellitus, erythrocytes surface changes in alcoholics.
13	*Ficus elastica* Roxb. Ex. Hornem Moraceae	Rubber (NE)	Leaves are used in treating constipation, mumps, boils and cardiac weakness. Leaf- paste is applied on wounds and bruises. Bark-paste is administered for jaundice, gonorrhea, ulcers and excessive urination.
14	*Hibiscus rosasinensis* L. Malvaceae	Mandara (NE)	Plant parts are used to cure liver disorders, control high blood pressure. Decoction of leaves, root and fruits are helpful in treatments of arthritis, boils and coughs, and the fruit is used externally in cases of wounds and ulcers.
15	*Ixora coccinea* L. Rubiaceae	Rangani (NE)	It is used for wound healing, anti-diarrhoeal and anti-inflammatory problems. It is used as haemostatic, antioxidant and anti-ulcerative properties.
16	*Leueas aspera* Spreng. Lamiaceae	Gayasha (E)	Leaf juice is poured into ear to retrieve ear pain and sores. Leaf juice is taken orally to cure complications due to non-poisonous snakebites. Leaves are rubbed on skin to get relief from itching sensation due to contact of caterpillars.
17	*Mangifera indica* L. Anacardiaceae	Amba (E)	The leaves are used as anti-diabetic, antioxidant, anti-inflammatory, antiviral, hepato-protective, hypoglycemic, anti-allergic and anti-cancerous remedy.
18	*Murraya koenigii* (L.) Spreng. Rutaceae	Vrusunga (E)	Plant is used for cough, sour eructation, burning sensation, pruritis, skin diseases, anorexia, dyspepsia, colic, flatulence, diarrhoea, dysentery, vomiting, stomatitis and ulcers. It has anti-diabetic and antioxidant effects.

19	*Musa sapientum* L. Musaceae	Kadali (E)	Plant sap is used to cure different types of eye infection
20	*Nyctanthes arbor-tristis* L. Oleaceae	Gangasiuli (NE)	Plant is used to cure inflammation, sciatica, rheumatism, dyspepsia, cough, asthma, constipation, hemorrhoids, baldness, premature graying of hair and pruritus. Useful part: leaves, flowers and seeds.
21	*Pisum sativum* L. Fabaceae	Matara (E)	Leaf paste is taken orally as contraceptive.
22	*Plumbago indica* L. Plumbaginaceae	Raktachita (NE)	Plants are used in digestive problem, rheumatism and paralysis.
23	*Plumeria rubra* L. Apocynaceae	Katha champa (NE)	Leaves are used in cough, ulcers, skin diseases, inflammations, arthritis and constipation.
24	*Polianthes tuberosa* L. Amaryllidaceae	Tuberose (NE)	The bulbs are dried, powdered and are used as a remedy for gonorrhoea. The bulb is rubbed up with turmeric and butter and applied to neonates to remove small red pimples on their bodies.
25	*Pongamia pinnata* L. Fabaceae	Karanja (NE)	Seed oil is used in coetaneous disease like scabies, herpes and leucoderma.
26	*Psidium guajava* L. Myrtaceae	Pijuli (E)	Leaves are used in wounds, ulcers, rheumatism, and leaves are chewed to relieve toothache. It has been effective in checking vomiting and diarrhoea in cholera patients. It is also applied on skin diseases. A combined decoction of leaves and bark is given to expel the placenta after childbirth.
27	*Punica granatum* L. Punicaceae	Dalimba (E)	It is widely used in treating certain types of cancer including leukemia, breast, prostate and colon cancer, dysentery, diarrhoea, excessive bleeding, expelling intestinal worms and parasites.
28	*Ricinus communis* L. Euphorbiaceae	Castor (E)	It is used to induce labor pain, stimulate lactation, arthritis, contraceptive when applied inside the vagina and on eye lids to soothe irritation.
29	*Syzygium cumini* (L.) Skeels Myrtaceae	Jamukoli (E)	Leaves and bark are used for controlling blood pressure and gingivitis.
30	*Thevetia neriifolia* Pers. Apocynaceae	Kaniar (NE)	Leaves are used as purgative and emetic.

Table 1: Ethnomedicinal information of 30 plants used.

Sl. no	Flavonoids	Saponins	Phlobatannis	Resins	Sterols	Lipids/ Fats	Steroids	Tannins	Glycosides	Acidic compounds	Terpenoids	Reducing sugar	Phenols	Carbohydrates	Anthraquinones
1	+ (+)	+ (-)	- (-)	- (-)	+ +)	- (-)	- (+)	- (-)	+ (-)	- (+)	+ (+)	- (+)	- (-)	- (-)	- (-)
2	- (-)	+ (-)	- (-)	+ (-)	- (-)	+ (-)	- (+)	+ (+)	+ (+)	- (-)	+ (+)	- (-)	+ (-)	- (-)	- (-)
3	- (+)	+ (+)	- (-)	- (-)	- (-)	+ (-)	- (-)	- (+)	- (-)	- (+)	- (-)	- (+)	- (-)	- (-)	+ (+)
4	- (-)	+ (-)	- (-)	- (+)	- (+)	+ (-)	+ (-)	+ (-)	- (-)	+ (-)	- (-)	- (-)	+ (-)	- (-)	- (-)
5	- (-)	- (-)	+ (-)	+ (-)	- (-)	+ (-)	+ (+)	- (-)	- (-)	+ (+)	+ (+)	- (-)	+ (-)	- (-)	- (-)
6	- (+)	+ (+)	+ (-)	+ (-)	+ (-)	- (-)	+ (+)	+ (+)	+ (+)	- (-)	+ (+)	- (+)	+ (-)	- (+)	+ (+)
7	- (-)	+ (-)	- (-)	- (-)	+ (-)	+ (+)	+ (-)	+ (-)	- (-)	- (-)	- (-)	+ (-)	+ (-)	- (-)	+ (-)
8	- (-)	+ (-)	+ (-)	+ (-)	- (-)	- (-)	+ (-)	+ (+)	- (-)	- (+)	+ (-)	+ (+)	+ (-)	- (-)	+ (-)
9	- (-)	+ (+)	+ (-)	+(+)	- (+)	- (-)	- (+)	+ (-)	+ (+)	- (-)	- (-)	- (-)	+ (-)	- (-)	- (+)
10	- (-)	+ (-)	+ (-)	+ (-)	- (+)	+ (-)	+ (-)	+ (+)	+ (-)	+ (+)	+ (-)	- (-)	+ (-)	- (-)	+ (-)
11	- (+)	+ (-)	+ (-)	+(+)	- (-)	- (-)	+ (-)	+ (-)	- (+)	- (-)	- (-)	+ (+)	- (-)	- (-)	+ (-)
12	- (-)	+ (+)	+ (-)	- (-)	+ (+)	- (-)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)	- (-)	- (-)	+ (-)
13	- (+)	+ (-)	+ (-)	- (+)	- (-)	- (-)	+ (+)	- (+)	- (-)	- (+)	+ (-)	- (-)	- (-)	- (-)	+ (+)
14	- (-)	+ (-)	+ (-)	- (-)	- (+)	+ (-)	+ (-)	+ (+)	+ (-)	- (-)	- (-)	+ (+)	- (-)	- (-)	+ (-)
15	- (-)	+ (-)	- (-)	- (-)	+ (-)	+ (-)	+ (+)	+ (-)	- (-)	- (-)	- (-)	+ (-)	+ (-)	- (-)	+ (+)

16	- (-)	+ (+)	- (-)	- (-)	+ (-)	+ (-)	- (-)	- (-)	- (-)	- (-)	- (-)	+ (-)	+ (-)	- (-)	+ (-)
17	- (+)	+ (-)	+ (-)	- (-)	+ +)	- (-)	- (-)	+ (+)	+ (+)	- (-)	- (+)	- (-)	- (-)	- (-)	+ (-)
18	- (-)	- (-)	+ (-)	+ (-)	+ (-)	- (-)	+ (-)	+ (+)	- (-)	- (+)	- (-)	- (-)	+ (-)	- (-)	+ (+)
19	- (-)	+ (+)	+ (-)	+ (-)	+ (-)	- (-)	+ (+)	- (-)	- (+)	- (-)	- (-)	- (+)	+ (-)	- (-)	+ (-)
20	- (-)	+ (-)	- (-)	+ (+)	- (-)	- (-)	- (-)	+ (-)	+ (-)	- (-)	+ (-)	- (-)	- (-)	- (-)	+ (-)
21	- (+)	+ (-)	- (-)	- (-)	+ (+)	- (+)	+ (+)	+ (+)	+ (-)	+ (+)	+ (+)	- (-)	+ (-)	+ (-)	+ (-)
22	- (-)	+ (-)	- (-)	- (-)	+ (+)	+ (-)	+ (-)	+ (-)	+ (+)	- (-)	- (-)	+ (+)	- (-)	- (-)	+ (-)
23	- (-)	+ (-)	- (-)	- (-)	+ +)	- (-)	+ (+)	+ (+)	+ (-)	- (-)	+ (-)	- (-)	+ (-)	- (+)	+ (-)
24	- (-)	+ (-)	+ (-)	+(-)	+ (-)	- (-)	+ (+)	- (-)	- (+)	- (-)	+ (+)	- (+)	- (-)	- (-)	- (+)
25	- (-)	+ (+)	- (-)	+ (+)	- (+)	- (-)	+ (+)	+ (-)	+ (-)	+ (+)	- (+)	- (-)	- (-)	- (-)	+ (-)
26	- (+)	+ (+)	- (-)	+ (-)	+ (-)	- (+)	+ (+)	+ (+)	+ (+)	- (-)	- (-)	- (-)	+ (-)	- (-)	+ (-)
27	- (-)	+ (-)	- (-)	+(+)	+ (-)	- (-)	+ (+)	- (+)	- (+)	- (-)	- (+)	- (-)	+ (-)	- (-)	+ (-)
28	- (-)	+ (-)	+ (-)	- (-)	+ (-)	- (-)	+ (-)	+ (-)	+ (-)	+ (+)	- (-)	- (+)	- (-)	+ (-)	+ (-)
29	- (-)	+ (+)	- (-)	- (-)	+ (-)	- (-)	+ (+)	- (-)	+ (-)	+ (+)	- (-)	- (-)	- (-)	- (-)	- (-)
30	- (+)	+ (-)	+ (-)	- (+)	+ (-)	- (-)	+ (-)	+ (-)	- (-)	- (-)	- (-)	- (+)	+ (-)	- (-)	+ (-)

Table 2: Preliminary phytochemical analysis of aqueous and ethanol extracts of 30 plants.

Bacterium	MTCC No.	Agar media	Colony morphology
Acinetobacter sp.	1425	Nutrient agar	Colourless smooth, opaque, raised and pinpoint colonies.
		MacConkey agar	Colourless smooth, opaque, raised, NLF colonies
Citrobacter sp.	1658	MacConkey agar	Late LF colonies light pink after 48 h
Enterobacter sp.	2990	Blood agar	Small, round and pin -point colony.
		MacConkey agar	LF and mucoid colonies
Escherichia coli	443	Nutrient agar	Flat dry, irregular colonies
		MacConkey agar	LF, flat dry pink, irregular colonies
		EMB agar	Flat dry, irregular colonies, with metallic green colour
		CLED agar	translucent blue colonies
Klebsiella sp.	4031	MacConkey agar	LF, pink, mucoid colonies.
		CLED agar	Yellow and mucoid colonies
Proteus sp.	1771	Blood agar	Swarming colonies
		CLED	translucent blue colonies
Pseudomonas aeruginosa	1688	Nutrient agar,	Large, irregular opaque colonies, with bluish green pigment.
Salmonella sp.	733	MacConkey agar	NLF, colourless colonies
		XLD agar	Red colour, pinpoint colonies with black center
Serratia marcescens	**Na**	Nutrient agar	Large, round, enlarged orange coloured colonies
Shigella sp.	2957	Nutrient agar	Smooth, grayish/colour less, translucent colonies
		MacConkey agar	Pale and yellowish (NLF), No black centre
		XLD agar	Red without black centre

Table 3: Morphology and culture characters of clinically isolated Gram-negative bacteria along with MTCC strains.

MTCC: Microbial Type Culture Collection; Na: not available. EMB: Eosin methylene blue agar; XLD: Xylose lysine deoxycholate; CLED: cystine lactose electrolyte deficient medium; LF, lactose fermenting; NLF: Non-lactose fermenting.

Bacteria	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	Motility
Acinetobacter sp.	+	-	-	-	+	+	V	ND	-	M
Citrobacter sp.	+	-	-	+	-	+	-	K/A+ H_2S	+	M
Enterobacter sp	+	-	-	-	+	+	V	A/A	+	M
E. coli	+	-	+	+	-	-	-	A/AG	+	M
K. oxytoca	+	-	+	-	+	+	+	A/AG	+	NM
K. pneumoniae	+	-	-	-	+	+	+	A/AG	+	NM
P. vulgaris	+	-	-	+	-	V	+	K/A H_2S	+	M
P. mirabilis	+	-	+	+	-	V	+	K/A H_2S	+	M
P. aeruginosa	+	+	-	-	-	+	+	ND	+	M
S. boydii	+	-	V	+	-	-	-	K/A	+	NM
S. dysenteriae	+	-	V	+	-	-	-	K/A	+	NM
S. flexneri	+	-	V	+	-	-	-	K/A	+	NM
S. sonnei	+	-	-	+	-	-	-	K/A	+	NM
S. marcescens	+	-	-	-	+	+	-	KA	+	M
S. typhi	+	-	-	+	-	-	-	K/A+H_2S	+	M
S. paratyphi A	+	-	-	+	-	-	-	K/A	+	M
S. paratyphi B	+	-	-	+	-	+	-	K/A+H_2S	+	M

Table 4: Summary of results of biochemical tests of MDR Gram-negative bacteria.

+: positive; MR: methyl red; VP: Voges-Proskauer; TSI: triple sugar iron; -: negative; V: variable; A: acid; K: alkali; G: gas; H_2S: H_2S production; M: Motile; NM: non motile; ND: Not done.

Bacteria	Glucose	Lactose	Sucrose	Maltose	Mannitol
Acinetobacter sp.	-	-	-	-	-
Citrobacter sp.	A	LLF	A	A	A
Enterobacter sp.	A	A	A	A	A
E. coli	+	+	V	-	+
P. vulgaris	+	-	-	-	+
P. mirabilis	+	-	-	-	+
K. oxytoca	+	+	+	-	+
K. pneumoniae	+	+	+	-	+
P. aeruginosa	+	-	+	V	V
S. paratyphi A	A+G	-	-	A+G	A+G
S. paratyphi B	A+G	-	-	A+G	A+G
S. typhi	A	-	-	A	A
S. marcescens	A	A	A	A	A
S. boydii	A	-	-	A	A
S. dysenteriae	A	-	-	-	-
S. flexneri	A	-	-	A	A
S. sonnei	A	LLF	LSF	A	A

Table 5: Summary of results of carbohydrate fermentation tests of MDR Gram-negative bacteria.

A: acid; A+G: acid +gas; V: variable; LLF: late lactose fermentation; LSF: late sucrose fermentation; '+': positive; -: negative

Antibiograms of bacteria: Antibiograms of the isolated 17 GN isolates were done by using 17 antibiotics of 5 different classes, four aminoglycosides, three β-lactams, five cephalosporins, one carbapenem and four fluoroquinolones, as per the CLSI guidelines. From the antibiogram, it was recorded that *Acinetobacter* sp. was resistant to tobramycin, aztreonam, piperacillin, piperacillin/tazobactam, cefepime, cefoperazone, cefoperazone/sulbactam, ceftazidime, imipenem, ciprofloxacin, gatifloxacin, ofloxacin, whereas it was recorded as resistant to amikacin, gentamicin, netillin, ceftriaxone and levofloxacin. Likewise, *Citrobacter* sp. was found sensitive to three antibiotics, ceftriaxone, imipenem and levofloxacin while, it was resistant to rest 14 antibiotics. Of 17 bacteria, *E. coli* was susceptible to the maximum number of antibiotics, i.e., six antibiotics, amikacin, gentamicin, netillin, piperacillin/tazobactam, ceftriaxone and imipenem, whereas it was resistant to the rest 11 antibiotics used. On the other hand, *S. paratyphi* B was resistant to all the 17 antibiotics used in the study. Similarly, antibiotic susceptibility results of the rest GN bacteria were recorded (Table 6). The results obtained clearly suggested that, the isolated organisms were floridly MDR. Percent values of each of 17 pathogens resistant to individual antibiotics of 5 antibiotic groups were recorded (Table 7). *E. coli* had the highest 82% resistance value to piperacillin and ceftazidime, followed by 74% to aztreonam and ceftriaxone, 69% to ofloxacin and a least resistance percent value 17% to tobramycin. Likewise, *K. oxytoca* had the highest 87% resistance value to ceftazidime, followed by 83% to gentamicin, 82% to ceftriaxone and the least resistance percent value 21% to tobramycin. Similarly, the resistance percent values of the rest other GN pathogens with 17 antibiotics used were recorded (Tables 6 and 7).

Bacteria	Susceptibility to prescribed antibiotics -																
	Aminoglycosides				β-lactams			Cephalosporins					Carba penem	Fluoroquinolones			
	AK	GEN	NET	TOB	AT	PI	PIT	CPM	CPZ	CFS	CAZ	CTR	IPM	CIP	GAT	LE	OF
Acinetobacter sp.	S	S	S	R	R	R	R	R	R	R	R	S	R	R	R	S	R
Citrobacter sp.	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R
Enterobacter sp.	R	R	R	R	R	R	S	R	R	R	R	S	S	R	R	S	R
E. coli	S	S	S	R	R	R	S	R	R	R	R	S	S	R	R	R	R
K. oxytoca	S	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R
K. pneumoniae	R	S	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
P. vulgaris	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
P. mirabilis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
P. aeruginosa	R	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R	R
S. boydii	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. dysenteriae	S	S	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
S. flexneri	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R
S. sonnei	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. marcescens	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R
S. typhi	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
S. paratyphi A	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. paratyphi B	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R

Table 6: Antibiotic sensitivity pattern of MDR Gram-negative bacteria.

AK: amikacin; GEN: gentamicin; NET: netillin; TOB: tobramycin; AT: aztreonam; PI: piperacillin; P/T: piperacillin/tazobactam; CPM: cefepime; CPZ: cefoperazone; CFS: cefoperazone/sulbactam; CAZ: ceftazidime; CTR: ceftriaxone; IPM: imipenem; CIP: ciprofloxacin; GATI: gatifloxacin; LE: levofloxacin; OF: ofloxacin.

Bacteria	Susceptibility to prescribed antibiotics																
	Aminoglycosides				β-lactams			Cephalosporins					Carbapenem	Fluoroquinolones			
	AK	GEN	NET	TOB	AT	PI	PIT	CPM	CPZ	CFS	CAZ	CTR	IPM	CIP	GAT	LE	OF
Acinetobacter sp.	23	30	20	14	71	30	21	71	62	34	78	72	21	53	20	16	30
Citrobacter sp.	7	8	12	31	63	40	39	69	52	15	82	74	31	63	22	27	43
Enterobacter sp.	78	56	51	32	60	78	34	61	56	24	74	68	42	38	16	19	56
E. coli	24	36	32	16	74	82	25	54	62	21	82	74	38	34	19	21	69
K. oxytoca	63	83	62	21	61	87	28	57	71	21	89	82	56	54	71	39	72
K. pneumoniae	69	48	81	25	58	91	34	64	52	16	91	92	58	61	69	67	82
P. vulgaris	49	54	31	23	46	69	21	78	61	14	78	79	37	67	78	68	83
P. mirabilis	68	59	28	17	51	67	23	83	73	31	73	74	39	56	31	40	78
P. aeruginosa	58	63	69	71	69	63	31	81	69	32	91	92	32	49	78	51	45
S. boydii	21	31	19	46	21	88	21	65	59	16	82	71	29	42	51	41	41
S. dysenteriae	15	20	21	39	21	78	27	78	75	21	76	51	41	35	31	30	19
S. flexneri	16	41	31	41	23	76	18	71	81	25	68	61	30	38	28	41	41
S. sonnei	19	21	14	19	18	84	31	68	69	23	59	56	26	39	49	28	37
S. marcescens	23	21	21	41	19	71	27	62	68	17	78	69	23	41	49	29	36
S. typhi	31	21	31	37	20	85	29	71	65	14	62	68	31	37	51	21	45
S. paratyphi A	18	19	16	36	30	87	30	56	65	10	72	71	21	41	36	41	28
S. paratyphi B	16	18	19	45	32	69	29	63	67	32	67	68	15	12	42	21	27

Table 7: Percentage of resistance of antibiotics of MDR Gram-negative bacteria.

AK: amikacin; GEN: gentamicin; NET: netillin; TOB: tobramycin; AT: aztreonam; PI: piperacillin; PIT: piperacillin/tazobactam; CPM: cefepime; CPZ: cefoperazone; CFS: cefoperazone/sulbactam; CAZ: ceftazidime; CTR: ceftriaxone; IPM: imipenem; CIP: ciprofloxacin; GAT: gatifloxacin; LE: levofloxacin; OF: ofloxacin; -: not used

Antibacterial activity of plants : Antibacterial activity of aqueous and ethanol extracts of 30 plants were recorded by the agar-well diffusion method. A MDR strain of *Acinetobacter* sp. was recorded highly susceptible to both aqueous and ethanolic extracts of *Amorphophalus campanulatus, Carthamus ticntorius, Diosypros melanoxylon, Euphorbia cadufolia, Plumbago indica*, and *Psidium guajava*, whereas it was moderately susceptible to *Calotropis procera, Canna indica, Cedrus deodera, Combretum decandrum, Cucurbita maxima, Ficus elastica, Hibiscus rosasinensis, Polyanthes tuberosa* and *Punica granatum*. Further plants, *Ixora coccinea, Leuas aspira, Mangifera indica, Pisum sativum, Thevita neriifolia* registered significantly less antibacterial activity on the MDR Acinetobacter strain, whereas *Azadirachta indica, Carica papaya, Nycthanthes arboritis, Plumeria rubra, Pongamia pinata* and *Ricinus communis* had no growth inhibiting effect on Acinetobacter sp. Likewise, *P. aeruginosa* was found highly susceptible to both aqueous and ethanolic extracts of *C. procera, C. indica, C. papaya, C. ticntorius, C. maxima, H. rosasinensis, N. arboritis*, whereas was moderately susceptible to aqueous and ethanolic extracts of plants, *C. deodera, E. cadufolia, Ficus elastica, P. tuberosa, R. communis, T. neriifolia.* Plants, C. *decandrum, L. aspira, M. indica, Syzigium cumini* had least the antibacterial activity. Ethanolic extracts of plant, *A. indica, Murraya koenigii, Musa sapientum, P. rubra and P. pinnata* had moderate antibacterial activities over a MDR P. aeruginosa, whereas its corresponding water extracts did not register any antibacterial properties. Similarly, antibacterial properties of the rest 28 plants on all 17 MDR GN bacterial strains were recorded. In general, ethanolic extracts of used plants had better antibacterial potencies in comparison to their corresponding aqueous extracts (Tables 8a and 8b). A $\chi 2$-test was conducted between the effective aqueous and ethanolic extracts of 30 plants with taking the inhibition zone size values more than 8 mm as 1 and less than 8 mm as 0 (Tables 8a and 8b). The numbers 1 and 0 found in aqueous and ethanol for different bacteria were taken for $\chi 2$ -test and it was found that

with df =16, the calculated value was 20.50, whereas the tabulated value was 26.30 at p=0.05. Since the calculated value was less than the tabulated value, at p=0.05 level, the null hypothesis stating that there is no difference between observed and expected values is accepted, at p=0.05 level. Therefore, there was equal effectivity for both aqueous and ethanol extracts of 30 plants against all the 17 MDR GN bacteria.

Bacteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acinetobacter sp.	18(19)	0(0)	16(18)	16(19)	0(0)	19(21)	15(16)	18(19)	14(16)	16(17)	19(19)	18(19)	16(18)	14(16)	12(15)
Citrobacter sp.	0(12)	0(14)	0(0)	14(16)	18(18)	20(20)	0(0)	18(18)	16(17)	12(18)	18(0)	0(0)	16(0)	14(15)	0(0)
Enterobacter sp.	0(14)	0(0)	0(12)	14(0)	16(0)	21(0)	0(12)	14(16)	16(18)	14(16)	0(17)	0(0)	0(0)	0(0)	0(0)
E. coli	16(18)	0(0)	14(16)	16(18)	18(19)	19(19)	0(0)	12(14)	16(18)	19(18)	0(9)	0(0)	14(17)	14(16)	0(0)
K. oxytoca	0(18)	0(17)	0(16)	14(0)	16(12)	18(17)	0(17)	8(18)	10(19)	12(16)	19(19)	0(0)	16(17)	14(16)	0(0)
K. pneumoniae	16(19)	14(16)	14(17)	14(16)	12(14)	16(18)	0(0)	16(18)	22(19)	18(18)	18(19)	14(1)	16(18)	14(16)	0(12)
P. vulgaris	16(17)	0(18)	12(14)	14(16)	16(17)	18(19)	0(0)	18(17)	14(18)	16(19)	19(18)	8(12)	12(16)	12(14)	0(12)
P. mirabilis	0(0)	8(14)	0(14)	0(14)	12(15)	16(16)	0(0)	0(12)	0(14)	14(16)	21(23)	16(18)	12(14)	14(16)	0(12)
P. aeruginosa	14(17)	0(12)	20(20)	22(21)	18(19)	18(18)	16(12)	0(12)	12(12)	24(26)	18(19)	16(17)	16(19)	22(20)	0(14)
S. boydii	0(12)	0(14)	0(0)	0(0)	0(12)	16(15)	0(13)	0(16)	0(17)	12(17)	0(18)	0(0)	0(0)	18(21)	12(17)
S. dysenteriae	0(0)	0(14)	0(15)	0(18)	0(19)	22(24)	0(12)	0(13)	16(18)	14(16)	0(19)	0(13)	0(15)	0(16)	16(16)
S. flexneri	0(0)	0(12)	0(0)	0(14)	0(14)	20(21)	0(12)	0(13)	0(14)	0(12)	0(12)	0(14)	16(18)	18(18)	0(11)
S. sonnei	0(0)	0(12)	0(12)	0(12)	12(14)	18(19)	0(12)	0(14)	14(16)	0(17)	0(12)	0(14)	0(13)	0(12)	0(14)
S. marcescens	18(18)	12(16)	14(16)	0(0)	14(16)	8(12)	12(16)	12(14)	16(18)	12(16)	19(19)	0(0)	14(18)	15(16)	0(0)
S. typhi	12(14)	13 (17)	13(15)	12(12)	14(14)	18(13)	0(12)	14(16)	16(15)	12(14)	18(16)	0(12)	14(16)	12(14)	10(15)
S. paratyphi A	12(14)	14(16)	0(15)	0(12)	14(0)	16(14)	14(16)	12(16)	0(0)	0(0)	14(16)	16(18)	14(16)	16(18)	0(16)
S. paratyphi B	14(16)	0(14)	16(15)	18(21)	0(0)	17(18)	0(0)	0(0)	0(0)	16(18)	0(16)	0(1)	14(16)	16(17)	16(18)

Table 8a: Antibacterial activity of aqueous and ethanol extracts of selected plants by the agar well diffusion method against of clinically isolated Gram-negative bacteria as size of inhibition diameter (mm).

Numbers 1 to 15 are serial numbers of plants given in Table 1; Upper row of values are measurements of zones of inhibition due to water -extracts and lower values in parenthesis ( ) are due to ethanol-extracts.

Bacteria	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Acinetobacter sp.	14(15)	12(14)	14(16)	16(17)	0(0)	12(14)	18(18)	0(0)	16(18)	0(0)	19 (20)	16(18)	0(0)	18(19)	14(16)
Citrobacter sp.	14(16)	16(17)	14(16)	0(0)	0(9)	16(17)	18(18)	0(0)	12(16)	0(8)	18(19)	14(16)	0(12)	12(14)	14(16)
Enterobacter sp.	18(18)	18(18)	19(0)	0(12)	0(14)	16(13)	0(17)	0(16)	0(16)	16(17)	14(16)	12(14)	18(19)	17(18)	0(0)
E. coli	15(17)	14(18)	0(0)	0(12)	0(9)	16(18)	16(18)	12(16)	14(16)	0(0)	16(16)	19(17)	16(18)	18(19)	14(18)
K. oxytoca	16(16)	14(16)	12(16)	13(14)	0(0)	14(16)	15(16)	12(14)	12(14)	0(0)	0(12)	12(14)	10(14)	18(19)	12(14)
K. pneumoniae	14(18)	12(18)	14(16)	14(16)	0(8)	0(0)	16(16)	12(18)	16(17)	0(8)	18(19)	16(1)	14(16)	21(0)	18(19)
P. vulgaris	10(14)	12(16)	10(14)	0(12)	0(12)	15(1)	16(16)	15(16)	14(16)	0(14)	12(16)	12(14)	10(12)	12(16)	15(16)
P. mirabilis	16(18)	14(16)	0(12)	0(13)	0(14)	16(18)	17(19)	14(16)	19(18)	0(0)	14(16)	12(16)	0(0)	10(12)	16(17)
P. aeruginosa	14(12)	14(14)	12(15)	0(12)	18(19)	19(18)	21(22)	0(14)	16(17)	0(14)	18(19)	18(18)	16(17)	14(16)	16(18)
S. boydii	22(21)	20(22)	16(19)	0(12)	0(13)	0(12)	12(13)	12(14)	0(15)	0(12)	0(0)	17(18)	19(20)	14(16)	0(12)
S. dysenteriae	0(12)	14(16)	16(18)	0(15)	0(12)	16(18)	0(0)	0(0)	0(0)	0(0)	18(20)	19(21)	0(0)	18(21)	20(22)
S. flexneri	14(16)	18(17)	19(20)	0(12)	0(0)	0(0)	16(18)	0(0)	18(19)	0(0)	18(19)	14(16)	0(0)	0(12)	14(16)
S. sonnei	14(14)	12(14)	0(14)	0(13)	14 (19)	14(16)	22(23)	16(19)	0(14)	0(16)	16(18)	14(19)	0(0)	18 (18)	0(0)
S. marcescens	14(16)	16(17)	0(0)	12(16)	16(18)	22(23)	14(18)	0(0)	0(0)	18(18)	18(19)	0(15)	16(18)	0(0)	12(14)
S. typhi	8(18)	0(12)	12(13)	0(0)	14(16)	12(13)	14(15)	0(0)	10(13)	10(12)	11(12)	11(12)	10(12)	18(19)	16(18)
S. paratyphi A	0(14)	0(0)	16(18)	0(0)	13()	14(18)	16(19)	0(0)	16(18)	0(0)	14(16)	12(14)	0(0)	14(16)	18(19)
S. paratyphi B	18(19)	21(22)	19(21)	0(0)	0(0)	0(0)	12(14)	0(12)	0(14)	0(14)	16(18)	16(18)	0(12)	14(18)	0(12)

Table 8b: Antibacterial activity of aqueous and ethanol extracts of selected plants by the agar-well diffusion method against of MDR Gram-negative bacteria.

Numbers 16 to 30 are serial numbers of plants given in Table 1; Upper row of values are measurements of zones of inhibition due to water -extracts and values in parenthesis ( ) are due to ethanol-extracts.

Plants with most conspicuous antibacterial properties for each bacterium are presented (Tables 9a). Two independent Student's t-tests were conducted, one for number of bacteria controlled by each of water or ethanolic extract, while the second test was with number of effective plants against a bacterium, with same '30 plants 17 MDR bacteria' combination. In the first test, was conducted for each MDR bacterium (Table 9b), the degree of freedom (df) =30–1=29, the calculated t-value=3.52 was greater than the tabulated t-value=2.76, at p =0.01 level, rejecting the null hypothesis that 'both aqueous and ethanolic extracts were equally effective' at p =0.01 level. In other words, ethanolic extract was more effective than the corresponding aqueous extract of each plant in controlling MDR bacteria. Similarly, in the second t-test conducted between the numbers of effective aqueous or ethanolic extracts of 30 plants against individual clinically isolated MDR bacteria (Table 9b). With df, 17–1=16, the calculated t=2.83 is greater than the tabulated t=2.60 at p=0.02 level, the difference between effective aqueous and ethanol extract was highly significant at p =0.02 level; thus, it is stated that 'ethanolic extract were effective than aqueous extracts' is true in 99.98% cases.

MIC values of both ethanolic and aqueous extracts of all the effective plants against all the 17 GN bacteria were determined with the help of a 96-well micro-titre plate. It was found that with *Acinetobacter* sp., the minimum MIC value was 0.78 mg/mL Similarly other MIC values were calculate and documented (Tables 10a and 10b).

After determining MIC values from the 96 micro-titre plate, MBC values were determined by inoculating on nutrient agar plate from the cultured 96 micro-titre plate. In *Acinetobacter* sp. the minimum MBC value was 3.12 mg/mL by the aqueous extract of plant *A. campanulatus, A. indica, C. indica and C. tinctorius, N. arbor-tristis and S. cumini*. However, it was 0.78 mg/mL by ethanolic extract of *H. rosa-sinensis*. In *Citrobacter* sp., the minimum MBC value was 3.12 mg/mL Similarly other MBC values were calculated and documented (Tables 11a and 11b).

Plant number	Gram-negative bacteria	
	Aqueous extract	Ethanol extract
1	9	13
2	5	14
3	8	14
4	10	13
5	12	13
6	17	16
7	4	11
8	10	16
9	12	15
10	14	16
11	10	16
12	6	11
13	13	14
14	14	16
15	5	12
16	15	17
17	15	16
18	13	14
19	4	13
20	5	12
21	13	14
22	15	16
23	7	10
24	11	15
25	3	10
26	15	16
27	16	17
28	9	11
29	15	15
30	13	15
mean±sd	10.6±4.17	14.03±2.0812

Table 9a: Number of MDR Gram-negative and Gram-positive bacteria sensitive to aqueous and ethanolic extracts of 30 plant.

The Student's *t*-test was conducted (see text).

Bacteria	Water extract	Ethanol extract
Acinetobacter sp.	1,3,4,6,7,8,9,11,12,13,14,15,16,17, 23,24,25,26,27,28,29,30 (total 22)	1,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22, 23,24,25,26,27,28,29,30 (total 28)
Citrobacter sp.	4,5,6,7,8,9,11,13,14,16,17,23,24,25,26,27,28,29 ,30 (19)	2,4,5,6,7,8,9,10,14,16,17,18,20,21,22,23,24,25,26,27, 28,29,30 (23)
Enterobacter sp.	3,4,5,6,8,9,16,17,24,26,27,29,30 (13)	1,3,4,7,8,9,10,11,16,17,18,20,21,22,24,25,26,27,28,29,30 (21)
E. coli	4,6,8,9,16,17,18,21,23,24,25,26,27,28,29,30 (16)	1,3,6,7,8,9,10,11,16,17,19,20,21,22,23,24,25,26,27,28,29,30 (22)
K. oxytoca	4,6,8,9,11,13,14,16,17,23,24,27,28,29,30 (15)	1,2,3,6,7,8,9,10,11,13,14,16,17,18,19,21,22,23,24,26, 27,28,29,30 (24)
K. pneumoniae	1,3,4,6,8,9,11,12,13,14,16,17,23,24,26,27,28,29 ,30 (19)	1,34,6,8,9,10,11,12,13,14,15,16,17,18,19,20,22,23,24,25,26,27, 28,30 (26)
P. vulgaris	1,3,4,6,8,9,11,12,13,14,16,17,23,24,26,27, 28,29,30 (19)	1,2,4,6,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26, 27,28,29,30 (28)
P. mirabilis	2,5,6,10,11,12,13,14,16,17,23,24,26,27,28,29 , 30 (17)	2,3,4,5,6,8,9,10,11,12,13,14,15,16,17,18,19,20,21, 22,23,24,25,26,27,28,29,30 (28)
P. aeruginosa	1,3,4,6,7,9,11,12,13,14,15,16,17,23,24, 25,26,27,28,29,30 (21)	1,2,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20, 21,22,23,24,25,26,27,28,29,30 (29)
S. boydii	6,9,11,12,13,14,15,16,17,18,20,26,27,29,30 (total 15)	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,26,27,28,2 9,30 (24)
S. dysenteriae	7,13,14,16,17,22,24,25,27,28 (10)	2,4,5,6,7,8,9,10,11,12,13,14,16,17,18,19,22,24,25,27, 28,30 (23)
S. flexneri	5,6,9,12,16,17,20,22,23,26,27,29 (12)	2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25 ,26,27,29 (27)
S. sonnei	6,10,14,15,16,17,21,22,23,27,28,29 (12)	1,2,5,6,7,8,9,10,11.14,15,16,17,18,19,20,21,22,23,24,25,26,27,2 8,29,30 (26)
S. marcescens	1,2,3,4,6,7,8,9,10,11,13,14,16,17,18,25,26,27,28 ,29,30 (21)	1,2,3,4,6,7,8,9,10,11,13,14,16,17,18,20,21,22,25,26,27,28,29,3 0 (24)
S. typhi	1,2,3,4,5,6,7,8,9,11,12,13,14,15,16,17,22,25,26, 28,30 (21)	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,23,24,25,2 6,27,28,30 (28)
S. paratyphi A	1,3,4,6,7,8,9,11,12,13,14,15,16,17,23,24,25,26,2 7,28,29,30 (22)	1,3,4,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,2 6,27,28,29,30 (28)
S. paratyphi B	1,3,4,9,11,12,13,14,15,16,17,18,29 (13)	1,2,3,4,9,10,11,12,13,14,15,16,17,18,22,23,24,25,26,27,28,29,3 0 (23)
mean±Sd	16.88±3.5	25.41±2.55

Table 9b: Number of plant of leaf of water extract and ethanol extract sensitive to MDR Gram-negative bacteria.

The Student's *t*-test was conducted (see text)

Bacteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Acinetobacter sp.	3.12 (0.78)	- (-)	1.5 (0.78)	1.5 (0.78)	- (-)	0.78 (0.19)	25 (6.25)	25 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	6.25 (1.6)
Citrobacter sp.	- (0.78)	- (3.12)	- (-)	1.5 (0.78)	0.78 (0.19)	0.78 (0.19)	- (-)	12.5 (6.25)	6.25 (6.25)	25 (25)	25 (-)	- (-)	6.25 (-)	1.6 (0.39)	- (-)
Enterobacter sp.	- (0.78)	- (-)	- (0.78)	1.5 (-)	0.78 (-)	0.78 (-)	- (6.25)	12.5 (6.25)	6.25 (6.25)	2 (25)	- (25)	- (-)	- (-)	- (-)	- (-)
E coli	3.12 (0.78)	- (-)	1.5 (0.78)	1.5 (0.78)	0.78 (0.78)	0.78 (0.19)	- (-)	12.5 (6.25)	6.25 (6.25)	25 (25)	- (6.25)	- (-)	6.25 (0.78)	1.6 (0.39)	- (-)
K oxytoca	- (0.78)	- (3.12)	- (0.78)	1.5 (-)	0.78 (0.78)	0.78 (0.19)	- (6.25)	12.5 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	- (-)	6.25 (0.78)	6.25 (0.78)	- (-)
K. pneumoniae	3.12 (0.78)	3.12 (0.78)	1.5 (0.78)	1.5 (0.78)	0.78 (0.19)	0.78 (0.19)	- (-)	12.5 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	- (1.6)

P. vulgaris	3.12 (0.78)	- (3.12	1.5 (0.78)	1.5 (0.78)	0.78 (0.19)	0.78 (0.19)	- (-)	12.5 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	- (1.6)
P mirabilis	- (-)	3.12 (0.78)	- (0.78)	- (0.78)	0.78 (0.19)	0.78 (0.19)	- (-)	- (6.25)	- (6.25)	25 (25)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	- (1.6)
P. aeruginosa	3.12 (0.78)	- (6.25)	1.5 (0.78)	1.5 (0.78)	1.5 (0.78)	0.78 (0.19)	25 (6.25)	- (6.25)	6.25 (6.25)	6.25 (25)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	- (1.6)
S. boydii	- (0.78)	- (6.25)	- (-)	- (-)	- (0.78)	1.5 (0.78)	- (6.25)	- (6.25)	- (6.25)	25 (25)	- (6.25)	- (-)	- (-)	- (0.39)	6.25 (1.6)
S. dysenteriae	- (-)	- (3.12)	- (0.78)	- (0.78)	- (0.78)	0.78 (0.19)	- (6.25)	- (6.25)	6.25 (6.25)	25 (25)	- (6.25)	- (6.25)	- (0.78)	- (0.39)	6.25 (1.6)
S. flexneri	- (-)	- (3.13)	- (-)	- (3.13)	- (3.13)	0.78 (0.19)	- (6.25)	- (6.25)	- (6.25)	- (25)	- (6.25)	- (6.25)	6.25 (0.78)	1.6 (0.39)	- (1.6)
S. sonnei	- (-)	- (6.25)	- (0.78)	- (3.13)	0.78 (3.13)	0.78 (0.19)	- (6.25)	- (6.25)	6.25 (6.25)	- (25)	- (6.25)	- (6.25)	- (0.78)	- (1.6)	- (0.78)
S. marcescens	3.18 (0.78)	3.13 (6.25)	3.13 (0.78)	- (-)	1.5 (0.78)	1.5 (0.78)	25 (6.25)	25 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	- (-)	6.25 (0.78)	1.6 (0.39)	- (-)
S. typhi	3.12 (0.78)	3.12 (0.78)	3.12 (0.78)	3.12 (0.78)	1.5 (0.78)	0.78 (0.19)	- (6.25)	12.5 (6.25)	6.25 (6.25)	25 (25)	25 (6.25)	- (6.25)	6.25 (0.78)	1.6 (0.39)	6.25 (1.6)
S .paratyphi A	3.12 (0.78)	3.12 (0.78)	- (0.78)	- (0.78)	25 (-)	0.78 (0.19)	25 (25)	12.5 (6.25)	- (-)	- (-)	25 (6.25)	25 (6.25)	6.25 (0.78)	1.6 (0.39)	6.25 (1.6)
S. paratyphi B	3.12 (0.78)	- (0.78)	1.5 (0.78)	1.5 (0.78)	- (-)	0.78 (0.19)	- (-)	- (-)	- (-)	25 (25)	- (6.25)	- (6.25)	6.25 (0.78)	1.6 (0.39)	6.25 (1.6)

Table 10a: MIC values of cold leaf-extracts with aqueous and 80% ethanol against MDR Gram-negative bacteria (mg/ml).

Numbers 1 to 15 are serial numbers of plants given in Table 1; Upper row of values are measurements of zone of inhibition due to water -extracts and lower values in parenthesis ( ) are due to ethanol. - and (-): not determined. MIC: Minimum inhibitory concentration.

Bacteria	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Acinetobacter sp.	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	3.13 (0.78)	- (-)	3.13 (0.39)	3.13 (0.78)	- (-)	25 (12.5)	- (-)	1.6 (0.78)	1.5 (0.78)	- (-)	1.6 (0.78)	3.13 (1.6)
Citrobacter sp.	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (-)	- (0.78)	3.13 (0.39)	6.25 (0.78)	- (-)	25 (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	- (0.39)	1.6 (0.78)	3.13 (1.6)
Enterobacter sp.	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (0.78)	- (0.78)	3.13 (0.39)	- (0.78)	- (6.25)	- (12.5)	6.25 (6.25)	1.6 (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	- (-)
E. coli	3.13 (1.6)	3.13 (1.6)	- (-)	- (0.78)	- (0.78)	3.13 (0.39)	3.13 (0.78)	12.5 (6.25)	25 (12.5)	- (-)	- (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	3.13 (1.6)
K. oxytoca	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (-)	0.78 (0.78)	0.39 (0.39)	6.25 (0.78)	12.5 (6.25)	25 (12.5)	- (-)	- (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	3.13 (1.6)
K. pneumoniae	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	0.78 (0.78)	- (0.78)	- (-)	6.25 (0.78)	12.5 (6.25)	25 (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (-)	3.13 (1.6)

P. vulgaris	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (0.78)	- (0.78)	3.13 (3.13)	6.25 (0.78)	12.5 (6.25)	25 (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	3.13 (1.6)
P. mirabilis	3.13 (1.6)	3.13 (1.6)	- (3.13)	- (0.78)	- (0.78)	3.13 (0.39)	6.25 (0.78)	12.5 (6.25)	25 (12.5)	- (-)	1.6 (0.78)	1.5 (0.78)	- (-)	1.6 (0.78)	3.13 (1.6)
P. aeruginosa	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (0.78)	0.78 (0.78)	3.13 (0.39)	6.25 (0.78)	- (6.25)	25 (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	3.13 (1.6)
S. boydii	3.13 (1.6)	3.13 (1.6)	3.13 (3.13)	- (0.78)	- (0.78)	- (0.39)	6.25 (0.78)	12.5 (6.25)	- (12.5)	- (6.25)	- (-)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	- (1.6)
S. dysenteriae	- (1.6)	3.13 (1.6)	3.13 (3.13)	- (0.78)	- (0.78)	3.13 (0.39)	- (-)	- (-)	- (-)	- (-)	1.6 (0.78)	1.5 (0.78)	- (-)	1.6 (0.78)	3.13 (1.6)
S. flexneri	3.13 (1.6)	3.13 (1.6)	6.25 (3.13)	- (0.78)	- (-)	- (-)	12.5 (6.25)	- (-)	12.5 (6.25)	- (-)	1.6 (0.78)	1.5 (0.78)	- (-)	- (0.78)	3.13 (1.6)
S. sonnei	6.25 (1.6)	3.13 (1.6)	- (3.13)	- (0.78)	0.78 (0.78	3.13 (0.39)	3.13 (0.78)	12.5 (6.25)	- (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	- (-)	1.6 (0.78)	- (-)
S. marcescens	3.13 (1.6)	3.13 (1.6)	- (-)	0.78 (0.78)	0.78 (0.78)	3.13 (0.39)	3.13 (0.78)	- (-)	- (-)	6.25 (6.25)	1.6 (0.78)	- (0.78)	3.13 (0.39)	- (-)	3.13 (1.6)
S. typhi	3.13 (1.6)	- (1.6)	6.25 (3.13)	- (-)	0.78 (0.78)	3.13 (0.39)	3.13 (0.78)	- (-)	12.5 (6.25)	6.25 (6.25)	1.6 (0.78)	1.5 (0.78)	3.13 (0.39)	1.6 (0.78)	3.13 (1.6)
S. paratyphi A	- (1.6)	- (-)	6.25 (3.13)	- (-)	0.78 (-)	3.13 (0.39)	3.13 (0.78)	- (-)	12.5 (12.5)	- (-)	1.6 (0.78)	1.5 (0.78)	- (-)	1.6 (0.78)	3.13 (1.6)
S. paratyphi B	3.13 (1.6)	3.13 (1.6)	6.25 (3.13)	- (-)	- (-)	- (-)	3.13 (0.78)	- (6.25)	- (12.5)	- (6.25)	1.6 (0.78)	1.5 (0.78)	- (0.39)	1.6 (0.78)	- (1.6)

Table 10b: MIC values of cold leaf-extracts with aqueous and 80% ethanol against MDR Gram-negative bacteria (mg/ml).

Numbers 16 to 30 are serial numbers of plants given in Table 1; Upper row of values are measurements of zone of inhibition due to water -extracts and lower values in parenthesis ( ) are due to ethanol. - and (-): not determined. MIC: Minimum inhibitory concentration.

Bacteria	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Acineto-bacter sp.	6.24 (1.56)	- (-)	6.24 (1.56)	6.24 (1.56)	- (-)	1.56 (6.24)	50 (50)	50 (50)	50 (50)	50 (50)	50 (25)	50 (25)	25 (1.56)	3.12 (0.78)	25 (3.12)
Citrobac-ter sp.	- (1.56)	- (6.24)	- (-)	3.12 (1.56)	1.56 (3.12)	1.56 (1.56)	- (-)	25 (25)	12.5 (12.5)	50 (50)	50 (-)	- (-)	25 (-)	3.12 (0.78)	- (-)
Enterobac-ter sp.	- (1.56)	- (-)	- (1.56)	3.12 (1.56)	3.12 (-)	1.56 (-)	- (1.56)	25 (12.5)	12.5 (25)	50 (50)	- (50)	- (-)	- (-)	- (-)	- (-)
E. coli	6.24 (1.56)	- (-)	3.12 (1.56)	3.12 (1.5)	1.56 (1.56)	1.56 (1.56)	- (-)	25 (12.5)	12.5 (25)	50 (50)	- (12.5)	- (-)	12.5 (1.56)	3.12 (0.78)	- (-)
K. oxytoca	- (1.56)	- (6.24)	- (1.56)	3.12 (-)	1.56 (1.56)	1.56 (1.56)	- (12.5)	25 (25)	12.5 (25)	50 (50)	50 (25)	- (-)	50 (1.56)	12.5 (1.56)	- (-)
K. pneu-moniae	6.24 (1.56)	6.24 (1.56)	3.12 (1.56)	3.12 (1.56)	1.56 (1.56)	1.56 (1.56)	- (-)	25 (12.5)	12.5 (12.5)	50 (50)	50 (12.5)	50 (25)	12.5 (1.56)	3.12 (0.78)	- (3.12)

P. vulgaris	6.24 (1.56)	- (6.24)	3.12 (1.56)	1.5 (1.56)	1.5 (1.56)	1.56 (1.56)	- (-)	25 (12.5)	12.5 (12.5)	50 (50)	12.5 (12.5)	50 (12.5)	12.5 (1.56)	3.12 (0.78)	- (3.12)
P. mirabilis	- (-)	6.24 (6.24)	- (1.56)	- (1.56)	1.5 (3.12)	1.56 (3.12)	- (-)	- (12.5)	- (12.5)	50 (50)	50 (25)	50 (25)	12.5 (1.56)	3.12 (0.78)	- (3.12)
P. aeruginosa	6.24 (1.56)	- (12.5)	3.12 (1.56)	3.12 (1.56)	3.12 (1.56)	1.56 (0.78)	50 (25)	- (12.5)	25 (12.5)	12.5 (50)	50 (12.5)	50 (12.5)	25 (1.56)	3.12 (0.78)	- (3.12)
S. boydii	- (1.56)	- (12.5)	- (-)	- (-)	- (6.26)	1.56 (1.56)	- (12.5)	- (12.5)	- (12.5)	50 (50)	- (12.5)	- (-)	- (-)	- (0.78)	12.5 (3.12)
S. dysenteriae	- (-)	- (6.26)	- (6.26)	- (6.26)	- (6.26)	1.56 (0.78)	- (12.5)	- (12.5)	12.5 (12.5)	50 (50)	- (12.5)	- (-)	- (-)	- (3.12)	12.5 (3.12)
S. flexneri	- (-)	- (6.25)	- (-)	- (6.25)	- 6.26	1.56 (0.78)	- (12.5)	- (12.5)	- (50)	- (12.5)	- (12.5)	12.5 (25)	6.26 (1.56)	- (3.12)	- (3.12)
S. sonnei	- (-)	- (12.5)	- (1.56)	- (6.25)	1.5 (6.25)	1.5 (3.12)	- (12.5)	- (12.5)	12.5 (25)	- (50)	- (12.5)	- (12.5)	- (1.56)	- (3.12)	- (3.12)
S. marcescens	6.24 (1.56)	6.24 (12.5)	6.24 (1.56)	- (-)	3.12 (1.56)	3.12 (1.5)	50 (25)	50 (12.5)	12.5 (12.5)	50 (50)	50 (12.5)	- (-)	12.5 (1.56)	3.12 (1.56)	- (-)
S. typhi	6.24 (1.56)	6.24 (1.56)	6.24 (1.56)	6.24 (1.56)	3.12 (6.25)	1.56 (3.12)	- (12.5)	25 (12.5)	12.5 (12.5)	50 (50)	50 (12.5)	- (12.5)	12.5 (1.56)	3.12 (0.78)	12.5 (3.12)
S, paratyphi A	6.24 (1.56)	6.24 (1.56)	- (1.56)	- (1.56)	50 (-)	1.56 (0.78)	50 (50)	25 (12.5)	- (-)	- (-)	50 (12.5)	50 (12.5)	12.5 (1.56)	3.12 (0.78)	12.5 (3.12)
S. paratyphi B	6.24 (1.56)	- (1.56)	3.12 (1.5)	3.12 (1.5)	- (-)	1.56 (3.12)	- (-)	- (-)	- (-)	50 (50)	- (12.5)	- (12.5)	12.5 (1.56)	3.12 (0.78)	12.5 (3.12)

Table 11a: MBC values of cold leaf-extracts with aqueous and 80% ethanol against MDR Gram-negative bacteria (mg/ml).

Numbers 1 to 15 are serial numbers of plants given in Table 1; Upper row of values are measurements of zone of inhibition due to aqueous-extracts and lower values in parenthesis ( ) are due to ethanol-extracts. MBC: Minimum bactericidal concentration.

Bacteria	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**
Acinetobacter sp.	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- -	6.26 (1.56)	6.26 (1.56)	- (1.56)	- (-)	50 (25)	- (-)	3.12 (1.56)	3.12 (1.56)	- (-)	3.12 (1.56)	6.26 (3.12)
Citrobacter sp.	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- (-)	- (1.56)	6.26 (0.78)	- (1.56)	- (12.5)	- (25)	12.5 (12.5)	3.12 (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	- (-)
Enterobacter sp.	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- (1.56)	- (1.56)	6.26 (0.78)	- (1.56)	- (12.5)	- (25)	12.5 (12.5)	3.12 (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	- (-)
E. coli	6.26 (3.12)	6.26 (3.12)	- (-)	- (1.56)	- (1.56)	6.24 (0.78)	6.24 (1.56)	6.25 (12.5)	50 (25)	- (-)	- (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	6.26 (3.12)
K. oxytoca	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- (-)	1.56 (1.56)	0.78 (0.78)	12.5 (1.56)	25 (12.5)	50 (25)	- (-)	- (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	6.26 (3.12)
K. pneumoniae	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	1.56 (1.56)	- (1.56)	- (-)	12.5 (1.56)	12.5 (1.56)	25 (12.5)	50 (25)	- (12.5)	3.12 (1.56)	6.26 (1.56)	6.26 (1.56)	6.26 (-)

P. vulgaris	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- (1.56)	- (1.56)	6.26 (6.26)	12.5 (1.56)	25 (12.5)	50 (25)	- (12.5)	3.12 (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	6.26 (3.12)
P. mirabilis	6.26 (3.12)	6.26 (3.12)	- (6.26)	- (1.56)	- (1.56)	6.26 (0.78)	12.5 (1.56)	25 (12.5)	50 (25)	- (-)	3.12 (1.56)	3.12 (1.56)	- (-)	3.12 (1.56)	6.26 (3.12)
P. aeruginosa	6.26 (3.12)	6.26 (6.26)	- (6.26)	1.56 (1.56)	6.26 (1.56)	12.5 (1.56)	- (12.5)	50 (25)	- (12.5)	3.12 (1.56)	3.12 (1.56)	6.26 (1.56)	6.26 (1.56)	6.26 (3.12)	6.26 (3.12)
S. boydii	6.26 (3.12)	6.26 (3.12)	6.26 (6.26)	- (1.56)	- (1.56)	- (0.78)	12.5 (1.56)	25 (12.5)	- (25)	- (12.5)	- (-)	6.26 (1.56)	6.26 (0.78)	3.12 (1.56)	- (3.12)
S. dysenteriae	- (3.12)	6.26 (3.12)	6.26 (6.26)	- (1.56)	- (1.56)	6.26 (0.78)	- (-)	- (-)	- (-)	- (-)	3.12 (1.56)	1.56 (1.56)	- (-)	3.12 (1.56)	6.26 (3.12)
S. flexneri	6.26 (3.12)	6.26 (3.12)	12.5 (6.26)	- (1.56)	- (-)	- (-)	25 (12.5)	- (-)	25 (12.5)	- (-)	3.12 (1.56)	3.12 (1.56)	- (-)	- (1.56)	6.26 (3.12)
S. sonnei	12.5 (3.12)	6.26 (3.12)	- (6.26)	- (1.56)	1.56 (1.56)	6.26 (0.78)	1.56 (1.56)	25 (12.5)	- (25)	- (12.5)	3.12 (1.56)	3.12 (1.56)	- (-)	3.12 (1.56)	- (-)
S. marcescens	6.26 (3.12)	6.26 (3.12)	- (-)	1.56 (1.56)	1.56 (1.56)	6.26 (0.78)	6.26 (1.56)	- (-)	- (-)	12.5 (12.5)	3.12 (1.56)	- (1.56)	6.26 (0.78)	- (-)	6.26 (3.12)
S. typhi	6.26 (3.12)	- (3.12)	12.5 (6.26)	- (-)	1.56 (1.56)	6.26 (1.56)	6.26 (1.56)	- (-)	25 (12.5)	12.5 (12.5)	3.12 (1.56)	3.12 (1.56)	6.26 (0.78)	3.12 (1.56)	6.26 (3.12)
S, paratyphi A	- (3.12)	- (-)	12.5 (6.26)	- (-)	1.56 (-)	6.26 (0.78)	6.26 (1.56)	- (-)	25 (25)	- (-)	3.12 (1.56)	3.12 (1.56)	- (-)	3.12 (1.56)	6.26 (3.12)
S. paratyphi B	6.26 (3.12)	6.26 (3.12)	12.5 (6.26)	- (-)	- (-)	- (-)	6.26 (1.56)	- (12.5)	- (25)	- (12.5)	3.12 (1.56)	3.12 (1.56)	- (0.78)	3.12 (1.56)	- (3.12)

Table 11b: MBC values of cold leaf-extracts with aqueous and 80% ethanol against MDR Gram-negative bacteria (mg/ml).

Numbers 16 to 30 are serial numbers of plants given in Table 1; Upper row of values are measurements of zone of inhibition due to aqueous-extracts and lower values in parenthesis ( ) are due to ethanol-extracts. MBC: Minimum bactericidal concentration.

Discussion

MDR pathogenic bacterial strains shiver down a hospital's spine by spreading nosocomial infections. Indeed, the available armamentaria with antimicrobial stewardship programme against MDR pathogenic bacteria are slowly narrowed/diminished [16,17], because of the slower rate of addition of newer antibiotics by apothecary. Plants remain the most tangible source of antimicrobials, as ethnomedicinal and folklore reports record age-old practices of the control of infectious diseases by aborigine/ethnic people all over, with herbal products. For example, antibacterial activities of the weed, *Argemone mexicana* were recorded against MDR *P. aeruginosa,* wherein leaf-extracts of the weed with ethanol, methanol and acetone had prominent antipseudomonad activity.15 Moreover, most ferns are non-edible plants, causing aversions to grazing animals; in a study, it was seen that the creeping fern, *Lygodium flexuosum* had a good control capacity over five MDR strains of GNs, *Enterobacter, Escherichia, Klebsiella, Proteus* and *Pseudomonas* [17]. In a study exclusively with ten MDR enteropathogens, ethanolic extracts of *Aegle marmelos, Holarrhena antidysenterica, Cassia fistula, Terminalia arjuna* and *Salvadora persica* registered remarkable *in vitro* antibacterial activities [18].

Furthermore, MDR strains of six uropathogens were checked for their susceptibility to 25 plants where, *A. marmelos, H. antidysenterica*, and additionally, *Withania somnifera* registered equally remarkable in vitro antibacterial activities [18]. MDR *A. baumannii* and *P. aeruginosa* strains were well controlled by the methanolic extract of the weed, *Lantana camara*, in a recent study (Dubey and Padhy 2013) [13]. Thus, crude phyto-extracts were seen amply controlling MDR strains of diverse pathogens, as conjectured from this and previous studies.

The exquisite stress of phyto-drugs as the natural mixture of different classes of compounds in a crude plant-extract is an unbreachable barrier; consequently, MDR bacteria however well-studded with the armamentaria of multidrug resistance, could not win over the crude extract of any plant generally, and specifically if extracts were from non-edible/poisonous plants. In this perspective, the non-committal attitude on crude phyto-drugs for the use as antimicrobials, but seeking pure phytochemicals only for the purpose, would be tantamount to the love for academic/scientific study only, but it would not be an attempt for an immediate practical solution in the crusade against the fast evolving MDR pathogens. However, the search for pure chemicals from phyto-drugs, as drugs should continue for the ultimate goal of holistic control of diseases. As has been seen from myriads of reports on antimicrobial activities of medicinal plants against drug sensitive/standard bacterial strains of culture collection centers that crude extracts invariably control bacterial strains *in vitro*. Thus, undermining crude phyto-extracts as drugs would decrease the credibility of medicinal plants and induce frenzy attitude against the drug targeting endeavour.

Antibiotic sensitive pathogens have a limited capacity of virulence as the employed antibiotic controls them *in vivo*. At a particular level, the host defense system also helps control of pathogens, when the later are in a smattering number. Indeed, for the internal protection, antibiotic producing organisms harbour antibiotic resistant genes in plasmids and chromosomes, as well as the associated transfer mechanisms [19,20]. Therefore, such genes and/or transposon must have been taken up, a priory, horizontally by the susceptible group of bacteria, via bacterial transformation and/or conjugation [21,22].

Moreover, bacteria having simple/plastic genomes undergo intrinsic (mutations) or acquire genetic (conjugations and transformation) changes in the presence of an antibiotic, as a stress factor from a drug resistant strain. As a result, accrual antibiotic resistance mechanisms are the clinical determinants of the pathogenesis. Indeed, the horizontal transfer of genetic materials from one organism to another appears faster than mutational changes, a phenomenon popularly called as 'evolution of quantum leaps [23]. Slowly, the use of more and more antibiotics for the control of infectious diseases, have led to multiple resistances, i.e., too many antibiotics are ineffective to progressively increasing resistant strains of pathogens, as if growth and momentum gained by a descending snow-ball, during the passage of time by mutation and acquisition of genes from related/unrelated bacteria, ending in shockingly repellant multidrug resistance. Older antibiotics slowly become obsolete, even the resistant mechanism against those are found in certain bacteria for which, those antibiotics were never applied. Drug resistant bacteria gain the capability of surviving and multiplying under antibiotic-stress conditions, confirming the biological rule, 'any limiting condition for the majority would be an excellent opportunity for the minority'. In the presence of a drug in a body *in vivo*, the progeny of a drug sensitive strain is eliminated and the resistant strain survives, multiplies as if developing from a doppelgänger, and predominates ultimately in causing a characteristic pathogenesis. It is because a suitable emulating agent for the control is absent, and if plant-based CAM would be present in parallel along with the employed antibiotic, there would be the coveted blithesome result.

MDR bacteria could be taken as if, the return of an enemy with extra strength (multiple resistance) after an earlier half-hurt by an antibiotic. Defenses produced by the host body sometimes are counteracted by the MDR marauding pathogen, as successful parasites live and reproduce to live — multiply for affecting pathogenesis. This has been demonstrated with *Salmonella enterica* serotype *typhimurium* [24]. Even, MDR *Neisseria gonorrhea* had been known to acquire MTR and SAP A MDR systems of genes, from *Salmonella enterica* serotype *typhimurium* [25,26].

Conclusion

Plants with most conspicuous antibacterial properties in controlling MDR strains of GN bacteria were aqueous and ethanolic extracts of plants, *Carthamus tinctorius, Cucurbita maxima, Murraya koenigii, Leucas aspera, Plumbago indica* and *Psidium guajava*. Similarly, aqueous and ethanolic extracts of plants *Ixora coccinea*, *Nyctanthes arbor-tristis, Polycythaemia rubra, Pongamia pinnata* and *Syzygium cumini* were the most effective against the isolated GP bacteria. Extracts of *Cedrus deodara, Musa sapientum* and *Euphorbia caducifolia* had the least antibacterial activity. In general, with

the ethanolic extracts, antibacterial activities were recorded better than with the corresponding aqueous extracts. It is dare to think of crude phyto-drugs to be used as CAM during empiric therapy in the treatment of an infectious disease from MDR bacteria. And crude extracts as CAM, if scaled up, could trigger business tycoons as antimicrobials, when the astonishing popularity of whole-plant concoctions in all nations is considered, holistically.

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