Monograph: *In vitro* efficacy of 30 ethnomedicinal plants used by Indian aborigines against 6 multidrug resistant Gram–positive pathogenic bacteria

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1. Introduction

A vast amount of literature has accumulated on the emergence and associated artifices of multidrug resistant (MDR) strains of all most all pathogenic bacteria[1]. It would be a mistake if alternate strategies for the control of MDR pathogens were not seriously pursued, as the development of dovetailed drugs from the mainstream medicine is comparatively slow. MDR pathogens often enforce patients to hospice at younger ages even, due to intractable infections at innards, leading to the slow onset of a terminal illness, that gruels emotionally till death. As the subject of the use of herbal drugs was suggested suitably in developed countries[2], corroborated by World Health Organization[3], the development of alternate therapy for the control of MDR bacteria is considered as the urgent need for apothecary (pharmacognosy, pharmacology and pharmaceutics)
who must prudently take up the associated endeavour. Thus, it is never a matter of choice rather than a matter of compulsion to lean to phyto-drugs, as those have been serving the humanity from the time immemorial. The modern medicinal system has several phyto-drugs in use such as, morphine, quinine, vinblastin, vincristine, atropine, digoxin, and taxol, to name a few in brief. Today, plants provide a 25% drugs prescribed worldwide, and 121 active compounds are in the current use for chemoprophylaxis. In addition, WHO recognizes 252 basic and essential drugs; an 11% drugs are exclusively from plant origin; a 60% of anti-tumor and anti-infectious drugs from natural origin are under clinical trials[3]. Thus, phyto-drugs gave good sense of promise to apothecary, from the vantage point of the inherent plethora of natural chemicals. Obviously, a vast majority of these drugs cannot be synthesized economically, and are obtained from wild and cultivated plant sources only. Further, a lot of pure phytocompounds have been developed and are used as drugs paradigmatically, by their own merit today[4]. By the by, the use of crude plant extracts is remarkably popular in consumerism. Furthermore, the primary benefits of plant–based medicines are comparatively sought after in tide of love for natural products rather than the synthetic ones, which have been well discussed in tubercle bacilli (TB) chemotherapy, elsewhere[5]. It is consensus that most plants have the history of traditional use as ethnomedicine; nevertheless, certain phytochemicals particularly from non–edible/ poisonous plants have unknown and known toxic effects that need be quantified pharmacologically before being used as drugs.

Indeed, drug resistance in pathogenic bacteria has been a commonplace of infection biology, for both Gram–negative (GN) and Gram–positive (GP) ones[6–7]. Virulent enteric bacteria, Klebsiella, Salmonella, Pseudomonas, Shigella, Escherichia and a few more are active in unhygienic, marginalized communities and urban–slum ghettos in developing countries on precipitating public health episodes[8]. In parallel several GP bacteria, MDR species of Staphylococcus, Enterococcus and Streptococcus have also created frightening situations due to the development of resistance to β-lactams and several other groups of antibiotics. For example, the methicillin resistant Staphylococcus aureus (S. aureus) (MRSA) had emerged with resistant to 95% of presently used antibiotics worldwide, and this particular bacterium, once known as a commensal inhabiting soft zones of human body, is now regarded as a superbug in the health domain for its unbridled notorious status of virulence. Indeed, its evolved strains have the multidrug resistance character by acquiring complicated acquired/mutational clonal nexuses. MRSA, vancomycin resistant S. aureus (VRSA), vancomycin intermediate S. aureus (VISA) have been the major causative organisms of morbidity and mortality due to acute surgical site infections and wound suppurations, everywhere. Particularly, the emergence of MRSA had led to the use of alternative drug, the clindamycin (macrolide) with blithe some control. However, the prevalence of the clindamycin resistant MRSA strain had also been reported from many laboratories, impeding smooth clinical management[9]. Inducible clindamycin resistance in MRSA, due to erythromycin resistance was reported from this hospital[10], challenging the hygienic condition of intensive care units (ICUs)[11]. Moreover, strains of S. aureus (MRSA, VISA and VRSA) have been prevalent at 20.83% in our hospital over a period of 30 months, ending in April 2012. Similarly, vancomycin resistant Enterococci (VRE) strains since 1982 till date worldwide have been slowly taken up a noteworthy momentum of spread, and VRE is regarded as the second–most prevalent GP pathogen in nosocomial settings[10, 12]. In our hospital as well, VRE and vancomycin sensitive Enterococci (VSE) strains were seen to be present in equal proportions, over a period of surveillance of 6 months[13]. Species of Enterococcus [Enterococcus faecalis (E. faecalis), Enterococcus avium, Enterococcus durans, Enterococcus faecium, Enterococcus gallinarum and Enterococcus solitarius] are primarily opportunistic pathogens and the intensive use of broad spectrum antibiotics has been attributed to the emergence of their MDR strains. For example, during surveillance in hospitals of the USA from 1999 to 2002, it was recorded that 9% of nosocomial blood stream infections were due to Enterococci; of them, 60% strains were VRE[14]. Most frequently, VRE colonizes gastrointestinal tract and skin. Incidentally, hospitalized patients and non–hospitalized workers as controls in a cattle–rearing area of France were known to have the asymptomatic carriage of VRE[15]. Further, many antimicrobials used for small animals (pets and food animals) are used in humans that promotes the transmission of bacteria to their owners. For example, drug–resistant strains of Staphylococcus intermedius, Campylobacter sp., Salmonella sp. and Escherichia coli (E. coli) had been cited as possible zoonotic concerns[16]. In dogs, E. coli strains were phylogenetically similar to pathogenic strains causing infection in human beings; more than 15% of canine fecal deposits in the environment contain E. coli strains related to virulent human strains, asymptotically[17]. Obviously, asymptomatic carriage of pathogenic bacteria may lead to the risk of development of severe infections in course of time when temporarily the body has a setback in the defense mechanism. Enterococcus sp. causes bacteremia, endocarditis, meningitis and diverticulitis[18]. Additionally, avoparcin, a glycopeptide antibiotic is used as a growth promoter in animal farming in Europe, and consequently avoparcin induced VRE had been prevalent. Surprisingly, one would hardly find a more vivid illustration of a commensal, transforming into a perilous pathogen with an armamentarium of multidrug resistance, in the last few decades, as S. aureus or E. coli is.

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 6 MDR GP pathogenic bacterial strains in vitro. Antibiograms of those isolated bacteria with 17 antibiotics of the day ascertained 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 complementary alternate medicine (CAM). 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 work[19], and for the possible use of these plants as CAM.

1.1. Literature survey of pathogenic bacteria

Frequent reports of resistance to the β–lactam group of antibiotics on GN bacteria comprising, enteropathogens, uropathogens and suppuratives have created a vacuum in the confidence of clinicians[20]. Sometimes, ICUs of hospitals have become cesspool–like dangerous places, since a patient admitted for one cause may often go back to community (society) with some newly acquired infectious pathogenic bacterium, which will cause to spread bacterium in community. This might well demonstrate in the surveillance of GN [Acinetobacter baumannii (A. baumannii), Pseudomonas aeruginosa (P. aeruginosa), Klebsiella pneumoniae] and GP (MRSA and MDR strains of Enterococci) bacteria[6, 21]. Eventually, the empirical use of antibiotics intended frequently for several infections becomes ineffective. As antibiotics have a pivotal role in clinical management today, it would be hard to think of an aspect of contemporary life that does not depend on antibiotics. Moreover, with a smattering of well–heeled and rich people in developing countries, the rise of clinical costs due to ailments from MDR bacteria can never be appropriated, as those have proved as notorious communal and nosocomial pathogens with diverse armamentaria of drug resistance[22].

Streptococci are another group of commensals; α–haemolytic species cause pneumonia and Streptococcus mutans (S. mutans) causes dental caries and endocarditis. The most common pathogen, Streptococcus pyogenes (S. pyogenes) (Group A streptococi) is a β–haemolytic species causing suppurrative infections, streptococcal pharyngitis and scarlet fever. Streptococcus agalactiae (Group B streptococci) causes pneumonia, meningitis and bacteraemia in neonates as well as in aged people with occasional systemic infections. These enteropathogens also infect the female genital tract, and cause premature rupture of membranes (PROM) during pregnancy and consequent neonatal infection ending in septicemia[23]. The γ–haemolytic strain of Streptococcus pneumoniae cause bacterial pneumonia, otitis media and sometimes leads to meningitis, and causes mortality in almost 1.6 million people every year, worldwide[24]. In the last 3 decades, resistance patterns of Streptococcus pneumoniae to β–lactams and macrolides have increased dramatically, worldwide[25].

Ferocious pandrug resistant (resistant bacteria to all antibiotics of the present time) are A. baumannii, Klebsiella pneumoniae, P. aeruginosa[26]. To control these pandrug resistant GN bacteria, a synergistic or combination therapy is often used with colistin (polymixin) and one from an older class of antibiotics, piperacillin or tazobactam, till date. In a study from Darjeeling (India), it was recorded that E. coli strains were resistant to almost all major antibiotics in present day; 20 antibiotics of different groups comprising three–generation cephalosporins were used in the study[27].

Further, under–5 child mortality from shigellosis had been estimated almost 99% of 165 million annual episodes[28], notwithstanding, governmental measures for the prevention and control of such fervent episodes. This grievous situation is frequently seen in elderly and immunocompromised people in the developing nations. Not surprisingly, empiric therapy with the first line antimicrobial drugs, ampicillin, trimethoprim/ sulfamethoxazole, chloramphenicol, nalidixic acid, co–trimoxazole and tetracycline against several infections were progressively ineffective due to bacterial multidrug resistance in developed nations[29, 30]. For MDR Shigella sp., the most preferred therapeutic options are fluoroquinolones for adults, oxyimino–cephalosporins for children. However in this study, resistance of 4 species of Shigella to ciprofloxacin were in the range 35% to 42% of isolated strains, for gatifloxacin, the resistance value was 28% to 78%, for levofloxacin, it was 28 –51%, for ofloxacin the value was 19 to 41%, for clinically isolated strains. Thus, the failure of fluoroquinolones created an average chance of 30% to 35% in the empirical therapy for Shigella sp. Of the three β–lactams, aztreonam was resistant to all the four Shigella sp. from 18% to 23%. Similarly, piperacillin/tazobactam was resistant by 18% to 31% while piperacillin was resistant by 78% to 88% of Shigella strains. Additionally, Shigella had extended spectrum β–lactamase (ESBL) producing clinical isolates in our study. Moreover, Shigella sonnei was reported to have the β–lactamase production by the plasmid mediation. Shigella sonnei had also been known as the predominant causative organism of enteric diseases in Asia, a priori for its production of chimeric β–lactamases, with the gene CTX–M–64[31]. Furthermore, quinolone–resistant E. coli strains were reported as widespread in Asia[32].

Studies on antibiotic–resistant mutants of enteropathogenic strains from Barcelona revealed that Campylobacter was the leading MDR pathogen, followed by Salmonella, Shigella and Yersinia[33]. In fact, Campylobacter and Salmonella are known to cause extra–intestinal pathogenesis, the latter causing UTI and abscess at various body parts–all leading to bacteremia. From an Indian study, it was reported that Shigella, Salmonella, Aeromonas sp. and Vibrio cholerae (V. cholerae) were the most frequently isolated pathogens from stools of under–5 children with diarrhea. Shigella was the most frequent pathogen, while non–typhoidal Salmonella and V. cholerae were in the decreasing trend of prevalence. Nalidixic acid, co–trimoxazole and furazolidone were resistant to Shigella, while V. cholerae strains were resistant to nalidixic acid and co–trimoxazole[34]. Shigella, Salmonella and E. coli were the most frequently isolated enteric pathogens in children of Ghana, among which, the majority isolates of E. coli and Shigella were resistant to methicillin, trimethoprim/ sulfamethoxazole and chloramphenicol[35].

The emergence of MDR strains of pathogens has many routes–drug efflux mechanisms operative at the plasma membrane level that is well demonstrated in E. coli and other pathogenic bacteria[36]. Secondly, extracellular and intracellular degradations of antibiotics are epitomized
by ESBL and carbapenamase producing GN bacteria; but
the development of drug resistance has not been properly
perceived. Thirdly, the drug resistant character probably
in R-plasmids or transposons are transferred from one
bacterium to the other; the receiving bacterium may be
even from a distant phylogeny; for example, the transfer
of the multiple antibiotic resistance locus from E. coli
had been demonstrated to be active in Mycobacterium
smegmatis[36].

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
sattering number. Indeed, for the internal protection,
antibiotic producing organisms harbour antibiotic
resistant genes in plasmids and chromosomes as well
as the associated transfer mechanisms[37,38]. 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[39,40].

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’[41]. 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. Therefore, 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 the reason
why a suitable emulating agent for the control is absent,
and if plant-based CAM were present in parallel along
with the employed antibiotic, there would be the coveted
blithesome result.

Moreover, the expression of antibiotic resistance in a
pathogen is co-expressed with virulence, demonstrated in E. coli
with the mar–locus (multiple antibiotic
resistance locus) that regulates the expression of 60
chromosomal genes, along with the expression of
multidrug resistance. The resultant clinical constellation
is the linkage between antibiotic resistance and the
expression of virulent gene regulation. It had been
demonstrated that antibiotic susceptibility of bacteria is
modulated by several factors, growth phase, pH, carbon
dioxide concentration, temperature, salt concentration,
and low iron content[42]. For example, in P. aeruginosa,
the quinolone treatment at a sub–inhibitory level induced
the expression of certain bacterial genes, which probably
should be expressive in quinolone–resistant strains of
the bacterium. Similarly, ciprofloxacin causes the Shiga–
toxin production by E. coli O157:H7 both in vitro and
in an animal model[43]. Thus, antibiotic stress induces
the expression of virulence at sub–lethal levels of the
challenging antibiotic. In such a situation, the use of a
combination therapy, with cognitively skilled with ‘two
antibiotics and two drugs’ at levels far below the mutant
preventive concentrations, at which those were non–
toxic to host, proved dangerous with Mycobacterium
tuberculosis by emergence of its MDR strains[5]. In
addition, it had been proved that quinolone–resistant S.
aureus, when treated with a sub–inhibitory concentration
of a quinolone increased the expression of fibroinectin–
binding proteins contributing to the emergence of the
virulent factor[44]. It was demonstrated that the
antibiotic treatment triggered the release of bacterial
products including endotoxin and lipopolysaccharide,
which improved its virulence character, and of related
pathogenic bacterial[45]. In addition, an exposure to low
concentration of antibiotics triggered the sigma factor,
which was linked to virulence, demonstrated in TB[46].
Therefore, the effect of antibiotics in a mixture of drug–
resistant and –sensitive pathogens is a complex matter
inducing several target and non–target effects. For
example, isoniazid–induced alterations in TB genome had
been recorded[47]. In such a situation, an application of
an antibiotic to a pathogen resistant to another antibiotic
of the same class of antibiotic promotes epidemics, since
the absence of a suitable/emulating control agent. A
synergistic use of CAM could be helpful in the situation.

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[48,49]. Even, MDR Neisseria
gonorrhoea had been known to acquire multiple
transferrable resistance regulator (mtrR) and sensitivity
to antimicrobial peptides A (SAP A) type MDR systems of
genomes, from Salmonella enterica serotype typhimurium[50].

β–lactam antibiotics are widely used as antibacterial
agents. When S. aureus was able to produce penicillinase
before 1960 after its introduction in 1940, methicillin was
introduced to combat penicillinase producing S. aureus
and MRSA was reported in 1961 itself[51]. Today, MRSA is
the most infamous GP pathogen of hospitals everywhere.
Methicillin resistance has arisen by the acquisition of
a novel DNA stretch, which results in the production of
newer penicillin binding proteins (PBP) namely, PBP2* or
PBP2a. In the meantime, around 2003, cephalosporins were
developed to control MRSA. PBP2* is a developed protein
and it is the product of 0.2 kb mecA gene; this is a part
of the larger mobile genetic elements in Staphylococci,
the staphylococcal chromosomal cassette mec. Around 5
different staphylococcal chromosomal cassette mec types
have been described, which vary in size from 0.2 kb to 0.7 kb[52]. Further, apart from the meca gene, complex genetic elements may contain integrated plasmids and transposons confirming resistance to other antibiotic classes. In fact, penicillinase production is common in S. aureus, but was rare in Enterococci, two decades ago[53], and up to that time it was not reported in other Streptococci even. Indeed, Enterococci are intrinsically resistant to marketed/available cephalosporins, as PBP has low affinity to cephalosporins for causing the degradation.

Transferable (vancomycin) glycopeptide resistance was first reported with the recognition of Enterococci with the gene VanA[54]. Other variants of glycopeptide resistant Enterococci (GRE) with VanB, VanC, VanD, VanE and VanG were reported subsequently[55]. GREs of all these types remain however, susceptible to all types of novel glycopeptides. Glycopeptides have a key role in serious infections in MRSA too. But, the development of VRSA and VISA had been recorded progressively leading to the spread of VRSA, worldwide. Teicoplanin (glycopeptide) was in use for the control of VISA/VRSA. But, both these glycopeptides were resistant to the present GP isolates. Moreover, Streptococci and Enterococci were insusceptible to gentamicin and other aminoglycosides due to poor transport across cytoplasmic membranes, a synergistic combination of derivatives of penicillin and aminoglycosides was used against these two pathogens elsewhere[56]. But, our isolates were amply resistant to two used aminoglycosides. Moreover, numerous mechanisms have been known for the resistance of GP bacteria to macrolides, lincosamides and streptogramin B agents (MLSb group)[57]. The MLSB group becomes resistant to MRSA by 23s rRNA methylases encoded by erm genes[57]. Efflux pumps are the major facilitators in the mechanism of macrolide resistance mediated by mef genes operated in Staphylococci. Other than erm and mef genes, a specific resistant pattern to lincosamides was reported in outlandish alleles of Staphylococci[57]. Further, fluoroquinolones target the resistance at the DNA–gyrase gene. Ciprofloxacin resistance has been shown to be mediated by both efflux mechanism and mutational target modifications. And the pumps involved in resistance in S. aureus are due to nor genes[58]. Thus the infection dynamics of MDR pathogens challenges the hygienic totem pole of a country; herein the artifices of ‘totally drug resistant TB’, reported from Italy and India are not considered. This clearly demonstrates the chicaning characteristics of MDR incarnations of pathogenic bacteria that need be controlled with an iron hand, and phytodrugs always remain as an affordable source of non-microbial antimicrobials, which are not yet adopted by the antimicrobial stewardship formally, despite the alarming situation of pathogen induced episodes in all hospitals, everywhere.

2. Materials and methods

2.1. Survey work

Plants reported (listed in Table 1, Figures 1–18) were collected from Kalahandi forest during December 2009; 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. The elevation ranges from 400 to 1200 m; temperature varies from 10 °C in winter to 46 °C in summer and the district experiences an average rain fall of about 128 cm and a rich biodiversity, typical to a sub-tropical forest. The survey was done with a questioner and personal interview, using the snowball technique in survey and sampling[19].

2.2. 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. Each mixture was hand–shaken at every 3–4 h, and filtered; filtrates were concentrated with the help of a rotary evaporator at 40 °C, till sticky masses were obtained, and those were incubated in a desiccator with fused calcium chloride for hardening sticky masses. These steps were repeated for each plant sample; 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.

2.3. Phytochemical analysis of plants

Plant extracts using ethanol and water were subjected to several chemical tests to know the presence of flavonoids, saponins, phlobatannins, resins, sterols, lipids/fats, steroids, tannins, glycosides, acidic compounds, terpenoids, reducing sugar, phenols, carbohydrates and anthra–quinones[59,60].

2.4. 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 agar was in the log phase of growth. The test bacterial strains isolated and used were the 6 GP bacteria belonging to 3 genera, S. aureus, Staphylococcus epidermidis (S. epidermidis), Staphylococcus saprophyticus (S. saprophyticus), E. faecalis, S. mutans, S. pyogenes isolated from clinical samples of patients of the outpatient department of Sum Hospital as well as, from patients admitted into ICUs, wards and cabins of the hospital, using an appropriate medium for a bacterium. These bacterial strains were isolated to pure (axenic) cultures before performing biochemical characterization[60].

2.5. Biochemical identifications and antibiotic sensitivity tests

For pure–cultures of isolated GP cocci, catalase and coagulase tests were performed. Catalase negative colonies were subjected to bile esculin test[13]. All the used 6 bacterial strains were subjected to antibiotic sensitivity test by the disc–diffusion/Kirby–Bauer’s
method, with 17 high potency antibiotic–discs (HiMedia), according to CLSI guidelines[61].

2.6. Antibacterial activity test by agar–well diffusion method

For monitoring antibacterial activity by the agar–well diffusion method, bacterial lawn was prepared. Wells were punched for 6 mm deep in 30 min old bacterial lawn and each well was based by 50 μL molten Muller–Hilton 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. 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[11,62].

2.7. MIC and MBC values of plant extracts against clinically 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 (12x8) micro–titer plate along with an aliquot of 100 μL nutrient NB (HiMedia, Mumbai), 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-plate test 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[11,62]. Experiment of each solvent extract was conducted thrice and results of the third repetition are presented.

### 3. Results

#### 3.1. 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 were 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. 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

### Table 2

| Sl. No. | Flavonoids | Saponins | Resins | Sterols | Lipids/Fats | Steroids | Tannins | Glycosides | Acidic compounds | Terpenoids | Reducing sugars | Phenols | Carbo- | 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      | - (-)      | + (+)    | - (-) | - (-)   | + (+)       | - (-)    | - (-)   | + (+)      | - (-)          | + (+)      | - (-)          | - (-)   | - (-)   |               |

No. corresponding to the sequence number in Table 1; Left column refer to aqueous extracts and values in parenthesis ethanol extracts; +: Present; -: Absent.

### Table 3

Morphology and culture characters of clinically isolated Gram–positive bacteria along with MTCC strains.

| Bacterium | MTCC No. | Agar media | Colony morphology |
|-----------|----------|------------|-------------------|
| *E. faecalis* | 439 | Nutrient agar | Oval cocci, in a pair arranged at an angle to each other |
|           |         | Blood agar  | Produces light–black colonies |
|           |         | Mannitol salt agar | Yellow colonies |
|           |         | MacConkey agar | Tiny deep pink colonies |
|           |         | Mannitol salt agar | Yellow colonies |
| *S. aureus* | 7443 | Nutrient agar | Golden yellow, opaque, circular colonies white butyrous consistency |
|           |         | Blood agar  | Beta haemolysis |
|           |         | Mannitol salt agar | Yellow colonies |
|           |         | Blood agar  | No growth, no haemolysis |
| *S. epidermidis* | NA | Nutrient agar | White, opaque, circular colonies |
|           |         | Blood agar  | No growth |
| *S. saprophyticus* | NA | Nutrient agar | White, opaque, circular colonies |
|           |         | Blood agar  | No growth |
| *S. mutans* | 497 | 6.5% NaCl broth | Growth |
|           |         | Blood agar  | Clear haemolysis around colony |
| *S. pyogenes* | 1928 | Blood agar | Colonies are small circular, semitransparent, low convex discs with an area of clear haemolysis around them |

MTCC: Microbial Type Culture Collections; NA: Not Available.
the antibacterial activities of plants. The results of phytochemical analyses of all plants are recorded (Table 2).

3.2. Bacterial identifications

GP bacteria as medium to large, smooth, entire, slightly raised, creamy yellow, with green/β-haemolytic colonies on blood agar, found positive to catalase, coagulase tests were confirmed as *S. aureus*. Catalase negative GP colonies showing β-haemolysis (complete haemolysis of erythrocytes) on blood agar and simultaneously sensitive to bacitracin were identified as Group A streptococci or *S. pyogenes*. Further, bile-esculin producing colonies, negative to catalase test were taken as *E. faecalis*, which produced greyish, round, small colonies with alpha-haemolytic zones on blood agar. Similarly, the rest three GP bacteria were identified (Tables 3 and 4, Figures 19–24).

### Table 5

| Bacteria          | Aminoglycosides | β-lactams | Cephalosporins | Fluoroquinolone | Glycopeptides | Macrolides | Lincomamide | Sulfonamide | Stand alone |
|-------------------|-----------------|----------|----------------|----------------|--------------|------------|-------------|-------------|-------------|
|                   | Ac   | Ge   | Ak   | Am   | Ox   | P    | Gtr | Cf   | Of   | Tei | Va | E   | Az   | Cd   | Cot | Ch   | Lz   |
| *E. faecalis*     | R    | R    | R    | S    | R    | R    | R   | R    | MS   | S    | S   | R   | S    | R    | R   | R    | S    |
| *S. aureus*       | R    | R    | S    | R    | R    | S    | R   | R    | S    | S    | S   | R    | S    | R    | R    | R    | S    |
| *S. saprophyticus*| R    | R    | R    | R    | R    | R    | R   | R    | MS   | R    | R    | S    | R    | R    | R    | R    | S    |
| *S. epidermidis*  | R    | R    | MS   | R    | R    | R    | R   | R    | S    | S    | R    | R    | S    | S    | S    | R    | R    |
| *S. mutans*       | R    | R    | R    | R    | R    | R    | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    |
| *S. pyogenes*     | R    | R    | R    | R    | R    | R    | R   | R    | R    | R    | R    | R    | R    | R    | R    | R    | R    |

| Ac: Amikacin (30 μg/disc); Ak: Amoxycilin (30 μg/disc); Am: Ampicillin (10 μg/disc); Az: Azithromycin (15 μg/disc); Cd: Clindamycin (2 μg/disc); Cf: Cefpodoxime (10 μg/disc); Ch: Chloramphenicol (30 μg/disc); C: Cot: Ceftriaxone (30 μg/disc); Ctr: Ceftriaxone (30 μg/disc); E: Erythromycin (15 μg/disc); Ge: Gentamicin (10 μg/disc); Lz: Linezolid (30 μg/disc); Of: Ofloxacin (5 μg/disc); O: Oxacillin (1 μg/disc); Pe: Penicillin (10 μg/disc); Tei: Teicoplanin (30 μg/disc); Va: Vancomycin (30 μg/disc).

### Table 6

| Bacteria          | Aminoglycosides | β-lactams | Cephalosporins | Fluoroquinolone | Glycopeptides | Macrolides | Lincomamide | Sulfonamide | Stand alone |
|-------------------|-----------------|----------|----------------|----------------|--------------|------------|-------------|-------------|-------------|
|                   | Ac   | Ge   | Ak   | Am   | Ox   | P    | Gtr | Cf   | Of   | Tei | Va | E   | Az   | Cd   | Cot | Ch   | Lz   |
| *E. faecalis*     | 67   | 38   | 42   | 37   | 73   | 62   | 72   | 52   | 46   | 40  | 23  | 45  | 54  | 25   | 43    | 69   | 32    |
| *S. aureus*       | 75   | 46   | 43   | 38   | 87   | 59   | 85   | 68   | 51   | 48  | 27  | 43  | 62  | 39   | 48    | 59   | 53    |
| *S. epidermidis*  | 62   | 32   | 22   | 27   | 17   | 48   | 68   | 28   | 23   | 16  | 3   | 27  | 45  | 29   | 37    | 48   | 43    |
| *S. saprophyticus*| 59   | 43   | 26   | 25   | 24   | 36   | 72   | 29   | 33   | 32  | 7   | 19  | 25  | 39   | 34    | 29   | 39    |
| *S. mutans*       | 69   | 58   | 52   | 42   | 38   | 57   | 68   | 32   | 48   | 38  | 17  | 23  | 42  | 35   | 52    | 38   | 33    |
| *S. pyogenes*     | 58   | 39   | 32   | 27   | 68   | 67   | 58   | 32   | 38   | 37  | 8   | 13  | 25  | 37   | 54    | 35   | 27    |

| Ac: Amikacin (30 μg/disc); Ak: Amoxycilin (30 μg/disc); Am: Ampicillin (10 μg/disc); Az: Azithromycin (15 μg/disc); Cd: Clindamycin (2 μg/disc); Cf: Cefpodoxime (10 μg/disc); Ch: Chloramphenicol (30 μg/disc); C: Cot: Ceftriaxone (30 μg/disc); Ctr: Ceftriaxone (30 μg/disc); E: Erythromycin (15 μg/disc); Ge: Gentamicin (10 μg/disc); Lz: Linezolid (30 μg/disc); Of: Ofloxacin (5 μg/disc); O: Oxacillin (1 μg/disc); Pe: Penicillin (10 μg/disc); Tei: Teicoplanin (30 μg/disc); Va: Vancomycin (30 μg/disc).

### Table 7

| Bacteria          | Catalase | Coagulase | Bile esculin | Novobiocin test |
|-------------------|----------|-----------|--------------|-----------------|
| *E. faecalis*     | ve       | ve        | ve           | Nd              |
| *S. aureus*       | ve       | ve        | Nd           | Sensitive       |
| *S. epidermidis*  | ve       | ve        | Nd           | Sensitive       |
| *S. saprophyticus*| ve       | ve        | Nd           | Resistant       |
| *S. mutans*       | ve       | ve        | ve           | Nd              |
| *S. pyogenes*     | ve       | ve        | ve           | Nd              |

- ve: Negative; +ve: Positive; Nd: not done.

3.3. Antibiograms of bacteria

Among the 6 GP bacteria, *E. faecalis* was found resistant to 10 antibiotics out of 17 antibiotics whereas, it was found sensitive to 6 antibiotics including ampicillin, oxacillin, teicoplanin, vancomycin, azithromycin and linezolid and moderately sensitive to ofloxacin. Likewise *S. aureus* was found resistant to 8 antibiotics out of 17 prescribed antibiotics whereas, it was sensitive to 8 antibiotics and moderately sensitive to one, azithromycin. Again, *S. pyogenes* was found resistant to all the 17 antibiotics used. Similarly, the antibiotic sensitivity pattern of the rest three GP bacteria were recorded (Table 5). Percent values of each of 6 GP pathogens resistant to individual antibiotics of 9 antibiotic groups were recorded (Table 6). *E. faecalis* had the highest 73% resistance value to oxacillin, followed by 72% to ceftriaxone, 69% to chloramphenicol and the least value 23% to vancomycin. Likewise, *S. aureus* had the highest 87% resistance value to oxacillin, followed by 85% to ceftriaxone, 75% to amikacin and the least resistance value 27% to vancomycin. Similarly, the resistance percent values of the rest other GP bacteria with all antibiotics used were recorded (Table 6).
3.4. Antibacterial activity of plants

Antibacterial activity of aqueous and ethanol extracts of 30 plants were tested by the agar–well diffusion method. A MDR E. faecalis strain was highly susceptible to the aqueous extracts of plants D. melanoxylon, N. arbor-tristis, P. indica, P. rubra and P. pinnata whereas, moderately susceptible to A. campanulatus, A. indica, C. indica, C. decandra, M. koenigii and M. sapientum. Aqueous extracts of the rest 19 plants were totally effective to control the MDR E. faecalis strain in vitro. Similarly, ethanolic extracts of C. indica, C. tinctorius, D. melanoxylon, I. coccinea, M. koenigii, P. rubra, P. pinnata and S. cumini were highly effective in controlling the E. faecalis MDR strain whereas, ethanolic extracts of the rest 22 plants were moderately effective. Likewise, the effectiveness both aqueous and ethanolic extracts of the 30 plants against the rest MDR 5 GP bacteria were recorded (Table 7).

Table 7

Antibacterial activity of aqueous and ethanol extracts of selected plants by the agar well diffusion method against MDR GP bacteria.

| Sl. No. | E. faecalis | S. aureus | epidermidis | saprophyticus | mutans | pyogenes |
|---------|-------------|-----------|-------------|--------------|--------|---------|
| 1       | 15 (13)     | 17 (14)   | 22 (19)     | 22 (20)      | 17 (20) | 19 (23) |
| 2       | 17 (19)     | 16 (19)   | 18 (23)     | 15 (26)      | 15 (19) | 18 (19) |
| 3       | - (16)      | - (-)     | 15 (19)     | - (16)       | 15 (18) | 18 (20) |
| 4       | 19 (23)     | 20 (19)   | 24 (20)     | 23 (22)      | 19 (18) | 19 (21) |
| 5       | - (17)      | 14 (22)   | 16 (19)     | 17 (26)      | 15 (20) | 15 (18) |
| 6       | - (21)      | - (18)    | 17 (21)     | - (22)       | 19 (18) | 18 (20) |
| 7       | - (19)      | - (14)    | - (18)      | - (12)       | - (15) | 15 (19) |
| 8       | - (15)      | - (-)     | 13 (17)     | 18 (22)      | - (17) | - (15) |
| 9       | 15 (19)     | 14 (18)   | 16 (20)     | 15 (27)      | 17 (15) | 12 (17) |
| 10      | - (16)      | - (-)     | - (16)      | - (-)        | - (19) | - (16) |
| 11      | 21 (26)     | 19 (21)   | 19 (26)     | 22 (25)      | 20 (24) | 23 (27) |
| 12      | - (15)      | - (19)    | 18 (20)     | 14 (20)      | - (-)  | - (14) |
| 13      | - (16)      | 12 (19)   | 12 (16)     | 17 (18)      | - (15) | - (17) |
| 14      | - (15)      | - (-)     | - (18)      | - (15)       | 15 (18) | - (-) |
| 15      | 24 (25)     | 23 (25)   | 24 (25)     | 24 (24)      | 23 (24) | 25 (26) |
| 16      | - (15)      | - (19)    | - (17)      | 15 (22)      | 15 (20) | 15 (19) |
| 17      | - (18)      | - (15)    | 14 (19)     | - (22)       | 12 (22) | 15 (20) |
| 18      | 16 (20)     | 12 (21)   | 15 (19)     | - (24)       | 19 (21) | 20 (25) |
| 19      | 16 (19)     | 15 (22)   | 14 (20)     | - (22)       | 15 (20) | 19 (22) |
| 20      | 22 (24)     | 25 (26)   | 24 (27)     | 25 (27)      | 24 (26) | 21 (23) |
| 21      | - (-)       | - (14)    | - (18)      | - (20)       | 15 (17) | 17 (20) |
| 22      | 26 (19)     | 24 (17)   | 36 (18)     | 33 (22)      | 18 (22) | 15 (20) |
| 23      | 24 (27)     | 23 (25)   | 24 (27)     | 22 (24)      | 21 (24) | 25 (26) |
| 24      | - (19)      | 15 (20)   | 18 (22)     | 16 (27)      | - (20) | - (17) |
| 25      | 24 (26)     | 23 (26)   | 21 (23)     | 20 (22)      | 21 (25) | 22 (24) |
| 26      | - (18)      | - (14)    | - (-)       | - (-)        | - (17) | - (18) |
| 27      | - (17)      | - (18)    | - (20)      | - (22)       | - (17) | - (17) |
| 28      | - (18)      | 14 (18)   | 14 (17)     | - (20)       | - (15) | - (17) |
| 29      | 25 (26)     | 22 (26)   | 20 (24)     | 21 (22)      | 21 (25) | 24 (27) |
| 30      | - (14)      | - (17)    | 12 (21)     | 15 (22)      | 16 (23) | 16 (19) |

Numbers 1 to 30 are serial numbers of plants given in Table 1; Left column of values are measurements of zones of inhibition due to aqueous extracts and values in parenthesis are due to ethanol extracts.

Plants with most conspicuous antibacterial properties for each bacterium are presented in Table 8. 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 6 GP MDR bacteria’ combination. The first test, was conducted for each MDR bacterium (Table 8), the $df=30-1=29$, the calculated $t$-value$=4.18$ was greater than the tabulated $t$-value$=3.66$, at $P=0.001$ level, rejecting the null hypothesis that ‘both extracts were equally effective’, at $P=0.001$ level. In other words, ethanolic extract was more effective than the corresponding aqueous extract of each plant in controlling 6 MDR GP bacteria. Similarly, the second $t$-test was conducted between the numbers of effective aqueous or ethanolic extracts of 30 plants against individual clinically isolated MDR bacteria (Table 9). With $df=6-1=5$, the calculated $t=6.9$ was greater than the tabulated $t=6.87$ at $P=0.001$ level, the difference between effective aqueous and ethanol extract was highly significant at $P=0.001$ level; thus, the statement that ‘ethanolic extracts were effective than aqueous extracts’ was true in 99.99% cases with GP bacteria.

Table 8

Number of plant of leaf of aqueous extract and ethanol extract sensitive to MDR GP bacteria.

| Sl. No. | Gram-positive bacteria | Aqueous extract | Ethanol extract |
|---------|------------------------|----------------|----------------|
| 1       | 6                      | 6              |
| 2       | 6                      | 6              |
| 3       | 3                      | 5              |
| 4       | 6                      | 6              |
| 5       | 5                      | 6              |
| 6       | 3                      | 3              |
| 7       | 1                      | 6              |
| 8       | 2                      | 5              |
| 9       | 6                      | 6              |
| 10      | 0                      | 4              |
| 11      | 6                      | 6              |
| 12      | 2                      | 5              |
| 13      | 3                      | 6              |
| 14      | 1                      | 4              |
| 15      | 6                      | 6              |
| 16      | 3                      | 6              |
| 17      | 3                      | 6              |
| 18      | 5                      | 6              |
| 19      | 5                      | 6              |
| 20      | 6                      | 6              |
| 21      | 2                      | 5              |
| 22      | 6                      | 6              |
| 23      | 6                      | 6              |
| 24      | 3                      | 6              |
| 25      | 6                      | 6              |
| 26      | 0                      | 4              |
| 27      | 0                      | 6              |
| 28      | 2                      | 6              |
| 29      | 6                      | 6              |
| 30      | 4                      | 4              |

Numbers 1 to 30 are serial numbers of plants given in Table 1; The student’s $t$-test was conducted (see text).
of 30 plants against 6 GP bacteria were determined. It was found that with S. aureus, the minimum MIC value was 0.39 mg/mL by the aqueous extract of N. arbor-tristis; while it was 0.39 mg/mL by ethanolic extracts of plants, N. arbor-tristis, P. rubra, P. pinnata, R. communis, S. cumini, and I. coccinea. With S. epidermidis, the minimum MIC value was 0.39 mg/mL by the aqueous extract of P. indica, S. cumini; while it was 0.39 mg/mL by ethanolic extracts of plants, D. melanoxylon, P. rubra, P. pinnata and S. mutans. With S. pyogenes, the minimum MIC value was 0.39 mg/mL by the aqueous extract of I. coccinea, while it was 0.39 mg/mL by ethanolic extracts of plants N. arbor-tristis, P. pinnata and S. cumini (Table 10).

### Table 10

| No. | S. aureus | S. epidermidis | S. saprophyticus | S. mutans | S. pyogenes |
|-----|----------|---------------|----------------|-----------|------------|
| 1   | 1.56 (0.78) | 0.78 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 2   | 1.56 (1.56) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 3   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 4   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 5   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 6   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 7   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 8   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 9   | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 10  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 11  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 12  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 13  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 14  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 15  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 16  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 17  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 18  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 19  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 20  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 21  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 22  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 23  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 24  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 25  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 26  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 27  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 28  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 29  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |
| 30  | 1.56 (0.78) | 1.56 (0.78)  | 1.56 (0.78)  | 1.56 (0.78) | 1.56 (0.78) |

Numbers 1 to 30 are serial numbers of plants given in Table 1; Left column of values are MIC of aqueous extracts and values in parenthesis are due to ethanol extracts.

MIC values of both ethanolic and aqueous extracts of 30 plants against 6 GP bacteria were determined. It was found that with S. aureus, the minimum MIC value was 0.39 mg/mL by the aqueous extract of N. arbor-tristis, while it was 0.39 mg/mL by ethanolic extracts of plants, I. coccinea, N. arbor-tristis and P. tuberosa. With F. faecalis, the minimum MIC value was 0.78 mg/mL by aqueous extracts of D. melanoxylon, I. coccinea, N. arbor-tristis, P. pinnata; while it was 0.39 mg/mL by ethanolic extracts of plants, I. coccinea, N. arbor-tristis, P. rubra and P. pinnata. With S. epidermidis, the minimum MIC value was 0.39 mg/mL by the aqueous extract of N. arbor-tristis; while it was 0.39 mg/mL by ethanolic extracts of plants, N. arbor-tristis, P. rubra, P. pinnata, R. communis, S. cumini, and I. coccinea. With S. saprophyticus, the minimum MIC value was 0.39 mg/mL by aqueous extracts of P. indica, S. cumini; while it was 0.39 mg/mL by ethanolic extracts of plants, D. melanoxylon, P. rubra, P. pinnata and S. mutans. With S. pyogenes, the minimum MIC value was 0.39 mg/mL by the aqueous extract of I. coccinea, while it was 0.39 mg/mL by ethanolic extracts of plants N. arbor-tristis, P. pinnata and S. cumini (Table 10).
MBC values of both ethanolic and aqueous extracts of 30 plants against all the 6 GP pathogenic bacteria were determined. It was found that with *S. aureus*, the minimum MBC value was 1.56 mg/mL by the aqueous extracts of *S. cumini*, *P. pinnata*, *P. rubra*, *P. indica*, *N. arbor-tristis*, while it was 0.78 mg/mL by ethanolic extracts of plants, *N. arbor-tristis*, *P. pinnata*, and *S. cumini*. With *E. faecalis*, the minimum MBC value was 0.78 mg/mL by the aqueous extract of *P. indica*, while it was 0.78 mg/mL by ethanolic extracts of plants, *S. cumini*, *P. pinnata*, *P. rubra* and *D. melanoxylon*. With *S. epidermidis*, the minimum MBC value was 1.56 mg/mL by aqueous extracts of *P. pinnata*, *P. rubra*, *N. arbor-tristis*, while it was 0.78 mg/mL by ethanolic extracts of plants, *D. melanoxylon*, *N. arbor-tristis*, and *P. rubra*. With *S. saprophyticus*, the minimum MBC value was 1.56 mg/mL by aqueous extracts of *N. arbor-tristis* and *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *A. indica*, *C. papaya*, *D. decandrum*, *N. arbor-tristis* and *P. tuberosa*. With *S. mutans*, the minimum MBC value was 1.6 mg/mL by aqueous extracts of *N. arbor-tristis*, *P. rubra*, *P. pinnata*, *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *P. rubra*, and *S. cumini*. With *S. pyogenes*, the minimum MBC value was 1.536 mg/mL by aqueous extracts of *I. coccinea*, *N. arbor-tristis*, *P. rubra*, *P. pinnata* and *S. cumini*, while it was 0.78 mg/mL by ethanolic extracts of plants, *D. melanoxylon*, *I. coccinea* and *N. arbor-tristis* (Table 11).

4. 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[1], since the slower rate of addition of newer antibiotics by apothecary.

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 local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care, plants, for the health-care needs of the numerically local forest-patch for all basic needs including the health care. The exquisite stress of phyto-drugs as the natural medicine (TM), derived from the clandestine ethnic information and Ayurveda[65]. 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’[66], TM as a field remains as the major accessible and affordable method of treatment for disease 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 CAM[67–69]. For example, 48% of population in Australia, 70% in Canada, 42% in USA, 38% in Belgium, and 75% in France use CAM, as it is known[3]; 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 person 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.

Moreover, most phyto–drugs cannot be ensconced for some ailments directly as morphine or quinine has been, but for host–toxicity testing. However, do we have the time for such studies with a myriad of phytochemicals, systematically and scientifically for the control of the avalanche of MDR pathogenic bacteria, spattering in both community and nosocomial settings in almost all countries? Logistically, the host–toxicity testing of crude phyto–drugs with mammals as antimicrobials needs be verified, but those do not serve the exact mirror of toxicity in man. Additionally, several attempts on monitoring of the synergistic effects of crude plant extracts with an obsolete antibiotic for the control of MDR bacteria in vitro have been undertaken[59]. In fact, a cohort of MDR enteric- and uro–pathogenic bacteria had been controlled in vitro by crude phyto–extracts from plants[8,11,70].

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 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[62]. 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[71]. 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[72]. Furthermore, MDR strains of six uropathogens were checked for their susceptibility to 25 plants where, Aegle marmelos, Holarrhena antidysenterica, and additionally, Withania somnifera registered equally remarkable in vitro antibacterial activities[72]. MDR A. baumannii and P. aeruginosa strains were well controlled by the methanolic extract of the weed, Lantana camara, in a recent study[11]. Thus, crude phyto–extracts were seen amply controlling MDR strains of diverse pathogens, as conjured from this and previous studies.

Plants with most conspicuous antibacterial properties in controlling MDR strains of GN bacteria were aequous and ethanolic extracts of plants, C. tinctorius, Cucurbita maxima, M. koenigii, L. aspera, P. indica and Psidium guajava. Similarly, aequous and ethanolic extracts of plants I. coccinea, N. arbor–tristis, P. rubra, P. pinnata and S. cumini were the most effective against the isolated GP bacteria. Extracts of C. deodara, M. sapientum and E. 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.

Conflict of interest statement
We declare that we have no conflict of interest.

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References
[1] Khan AU, Raffaele Z. Multidrug resistance: a global concern. Sharjah: Bentham Science; 2011.
[2] Sen S, Chakraborty R, De B. Challenges and opportunities in the advancement of herbal medicine: India’s position and role in a global context. J Herb Med 2011; 1: 67–75.
[3] WHO. WHO traditional medicine strategy 2011. WHO/EMP/ MIE/2011.2.3. Geneva: World Health Organization; 2011, p. 1–14.
Ghana. J Antimicrob Chemother 2008; 61: 1315–1318.

[36] McMurry LM, Levy SB. The periplasmic protein MppA is not involved in regulation of marA in Escherichia coli. Antimicrob Agents Chemother 2011; doi: 10.1128/AAC.05030-11.

[37] Davin-Regli A, Bolla JM, James CE, Lavigne JP, Chevalier J, Garnot E, et al. Membrane permeability and regulation of drug ‘influx and efflux’ in enterobacterial pathogens. Carr Drug Targets 2008; 9: 750–759.

[38] Mamelli L, Petit S, Chevalier J, Giglione C, Lieutaud A, Meinnel T, et al. New antibiotic molecules: bypassing the membrane barrier of Gram negative bacteria increases the activity of peptide deformylase inhibitors. PLoS One 2009; doi: 10.1371/journal.pone.0006443.

[39] Pagès JM, James CE, Winterhalter M. The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat Rev Microbiol 2008; 6: 893–903.

[40] Warnes SL, Highmore CJ, Kevil CW. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. mBio 2012; doi: 10.1128/mBio.00489-12.

[41] Groisman EA, Ochman H. Pathogenicity islands: bacterial evolution in quantum leaps. Cell 1996; 87: 791–794.

[42] Leekha S, Terrell CL, Edson RS. General principles of antimicrobial therapy. Mayo Clin Proc 2011; 86: 156–167.

[43] Menrath A, Wieler LH, Heidemanns K, Semmler T, Fruth A, Kemper N. Shiga toxin producing Escherichia coli and other negative bacteria. J Antimicrob Chemother 2011; doi: 10.1128/AAC.05030-11.

[44] McMurry LM, Levy SB. The periplasmic protein MppA is not involved in regulation of marA in Escherichia coli. Antimicrob Agents Chemother 2011; doi: 10.1128/AAC.05030-11.

[45] Sever DS, Alexander ME. Science, technology, and human factors in fire danger rating: the Canadian experience. Int J Wildland Fire 2005; 15: 121–135.

[46] De Silva T, Centre for Science and Technology of the Non-Alignment and Other Developing Countries. Traditional and alternative medicine: research and policy perspectives. New Delhi: Daya Publishing House; 2009.

[47] Dubey D, Rath S, Sahu MC, Debata NK, Padhy RN. Antimicrobial activity of Argemone mexicana L against multidrug resistant Pseudomonas aeruginosa, isolated from clinical samples. Asian Pac J Trop Biomed 2012; 2(Suppl 2): S846-S854.

[48] Rath S, Padhy RN. Monitoring my patient? Obstet Gynecol Clin North Am 2010; 37: 411–424.

[49] Rath S, Padhy RN. Monitoring in vitro antibacterial efficacy of Terminalia alata Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples. J Acute Med 2013; 3: 93–102.

[50] Rath S, Padhy RN. Monitoring in vitro antibacterial efficacy of Terminalia alata Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples. J Acute Med 2013; 3: 93–102.

[51] Rath S, Padhy RN. Monitoring in vitro antibacterial efficacy of Terminalia alata Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples. J Acute Med 2013; 3: 93–102.

[52] Rath S, Padhy RN. Monitoring in vitro antibacterial efficacy of Terminalia alata Heyne ex. Roth, against MDR enteropathogenic bacteria isolated from clinical samples. J Acute Med 2013; 3: 93–102.