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ORIGINAL ARTICLE

In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria

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Abstract Urinary tract infection (UTI) has become a more grievous problem today, due to multidrug resistance of infecting Gram-positive (GP) and Gram-negative (GN) bacteria, sometimes even with multiple infections. This study examines effectivity of 9 tropical flowering plants (*Anogeissus acuminata*, Azadirachta indica, *Bauhinia variegata*, *Boerhaavia diffusa*, *Punica granatum*, *Soymida febrifuga*, *Terminalia chebula*, *Tinospora cordifolia* and *Tribulus terrestris*) for possible use as source of antimicrobials for multidrug resistant (MDR) bacteria, along with main-stream antibiotics. Pathogenic bacteria were isolated from urine samples of patients attending and admitted in the hospital. Antibiograms of 11 isolated bacteria (GPs, *Enterococcus faecalis* and *Staphylococcus aureus*; and GNs, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa*) were ascertained by the disc-diffusion method, and antibacterial effectivity of plant extracts was monitored by the agar-well diffusion method. Isolated bacteria were floridly MDR to most antibiotics of the day. Methanol extracts of 9 plants were used, and extracts of 3 plants, *A. acuminata*, *P. granatum* and *S. febrifuga* at least caused 25–29 mm as the maximum size of zone of inhibition on bacterial lawns. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of methanol extracts of 9 plants were recorded. The methanol extract of *A. acuminata* had 0.29 mg/ml as the lowest MIC value and 0.67 mg/ml as the lowest MBC value, against MDR *S. aureus*, signifying effectivity; but, it had the highest MIC value

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of 3.41 mg/ml. and the highest MBC value of 4.27 mg/ml for most other MDR bacteria including *E. coli*. Qualitative phytochemical analysis was done for these 9 plants and information on leading phytochemicals was presented retrieved from PubChem database. Thus, three effective-most plants in controlling MDR-UTI bacteria *in vitro* were *A. acuminata*, *P. granatum* and *S. febrifuga*, *which can be promoted as* complementary medicine.

1. Introduction

Urinary tract infection (UTI) was mostly caused by Gram negative (GN) bacteria, predominately by *Escherichia coli* and by mixed infections of (Gram positive, GP) *Staphylococcus aureus*, *Enterococcus faecalis*, including other GNs, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter freundii*, *Proteus vulgaris* and *Klebsiella oxytoca* in the decreasing order of prevalence, when monitored in the Institute of Medical Sciences and Sum Hospital, for example (Mishra et al., 2013). The infecting bacteria normally constitute the faecal flora, and the UTI episode is initiated, when the urine flow in an individual is obstructed by one of several reasons such as, strictures, calculi, tumours, prostatic hypertrophy, vesicourethral reflux, diabetes, anal disease, pregnancy, catheterization, some surgical procedure at the urinogenital region and cystoscopy (Saint et al., 2002). The infecting bacteria invade urethra and bladder with a compromised body defense mechanism and decreased urine flow. In these conditions, the bacteria ascend via urethra move to the bladder mucosa, colonize, multiply and cause inflammation; this causes intolerable pain, burning, frequency and urgency of urination, nocturia, foul smelling, cloudy urine and haematuria. Indeed, at the onset of the problem, the patient reports to the physician and an empiric therapy is started before the culture report of the urine sample is obtained.

If any infection in a patient is not controlled, infecting microbes get resistance to the applied antibiotics intrinsically and a drug resistant cell survives and predominates with concomitant bacterial genetic exchanges mechanisms (McMurry and Levy, 2011). In short, there are several factors of antibiotic resistance in pathogenic bacteria and this situation has become a clinical consternation. A physician often prescribes some higher generation antibiotics in empiric therapy to avoid the debacle from treatment failure arising from the possible presence of MDR bacteria. And the UTI being a graver problem than imagined because of frequent attacks in females mainly, some complementary/adjuvant/synergistic therapeutic strategy is warranted.

Furthermore, information from ethnobotany/traditional medicine has been seen useful for several health complications other than infectious diseases. Nine flowering plants (*Anogeissus acuminata*, Azadirachta indica, *Bauhinia variegata*, *Boerhaavia diffusa*, *Punica granatum*, *Soymida febrifuga*, *Terminalia chebula*, *Tinospora cordifolia* and *Tribulus terrestris*) (Fig. 1) were used. These plants are in use traditionally by local ethnic tribes against infectious diseases; and these were examined in a systematic screening with UTI causing bacteria for use as source of non-microbial antimicrobials, so that these could serve as complementary medicine, along with mainstream drugs, the antibiotics, as it takes 3–4 days of time of arrival of the culture report of the urine during which period, infecting bacteria cause further problems. UTI episodes need to be controlled with an iron hand. And to avoid the use of any antibiotic of higher generation during empiric therapy, complementary or synergistic therapy using phyto-drugs with any ongoing antibiotic could be prudently used against UTI.

In continuation to previous work on scientific verification of ethnomedicinal information of a group of plants from Kalahandi (Odisha) forest (Mishra and Padhy, 2013; Rath and Padhy, 2014), the cited 9 plants were selected. These plants were too recorded in Indian pharmacopeia (Anonymous, 2014), as plants frequently used against general infectious diseases: *A. acuminata* is in use for UTI and skin diseases; whole plant of *A. indica* is used as an antiseptic; *B. variegata* is used against diarrhoea and throat infections; *B. diffusa* is used for UTI and dysentery; *P. granatum* is used for treating diarrhoea, dysentery and throat problems; *S. febrifuga* is used against diarrhoea, dysentery and UTI; *T. chebula* is used for diarrhoea and dysentery; *T. cordifolia* has anti-tubercular activity; and *T. terrestris* is used against UTI. Several other plants, *Terminalia alata*, *Lantana camara*, *Combretum albidum* and *Woodfordia fruticosa* had comparable antibacterial activities, which had been used for further pharmacognostical studies (Rath and Padhy, 2012; Rath and Padhy, 2013; Dubey et al., 2014; Sahu et al., 2014), but these 9 cited plants were not used for the isolation of individual compounds against any bacteria. Detailed antibacterial work with pathogenic bacteria isolated from clinical samples of patients in the hospital, with 9 plants is described.

2. Materials and methods

2.1. Collection of plants and extract preparation

Plants reported were collected from the *Kandha* tribe at hills of Eastern range of mountains of India, in the district Kalahandi, Odisha in January 2014. About 50 respondents of 25 hamlets were interviewed in a forest patch and the recorded information was documented (Table 1, Fig. 1), with the snowball method of survey and sampling. Methanol extracts of dried leaf samples dissolved in 10% v/v dimethyl sulfoxide (DMSO) were used, as detailed (Mishra and Padhy, 2013).

2.2. Isolation, identification of bacterial strains and antibiotic sensitivity test

Two GPs, *E. faecalis* and *S. aureus* including 9 GNs, *A. baumannii*, *C. freundii*, *E. aerogenes*, *E. coli*, *K. oxytoca*,

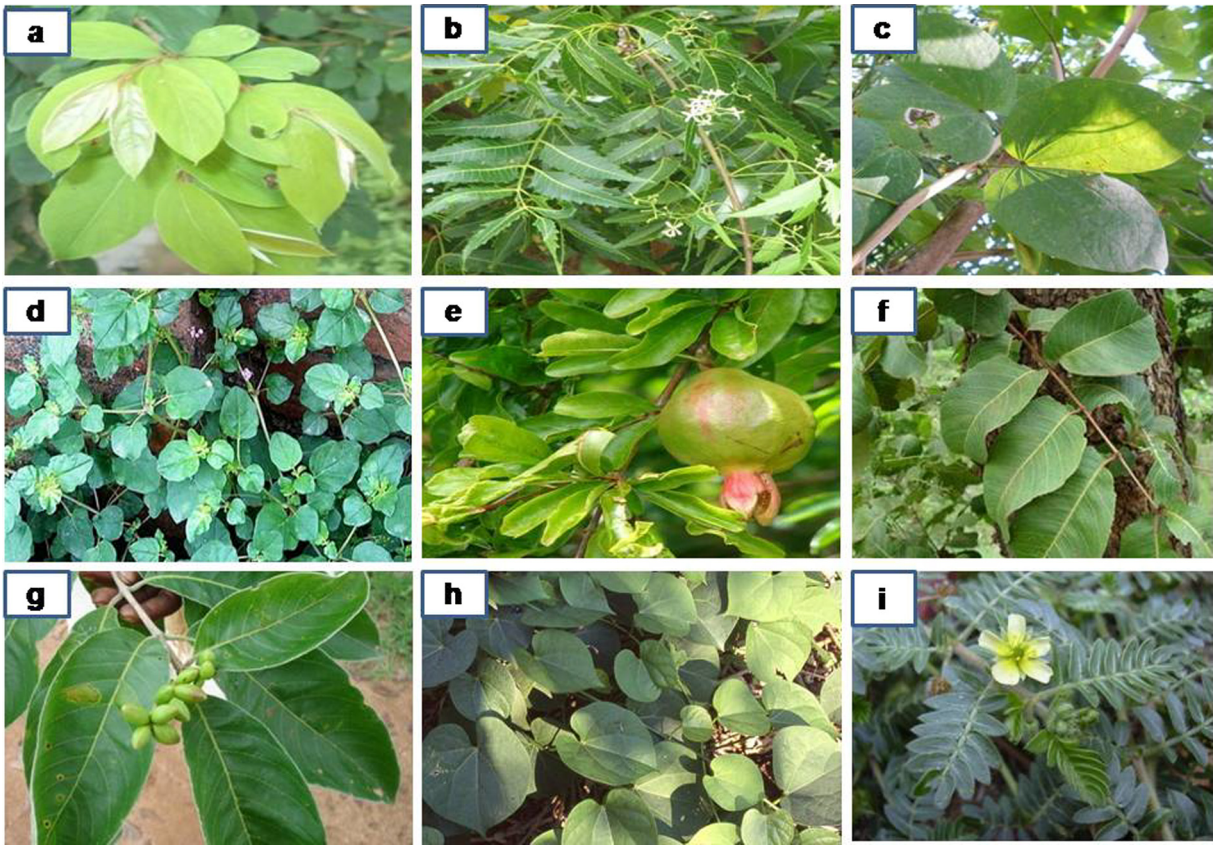


Figure 1 Photographs of plants: a, *Anogeissus acuminata*; b, *Azadirachta indica*; c, *Bauhinia variegata*; d, *Boerhaavia diffusa*; e, *Punica granatum*; f, *Soymida febrifuga*; g, *Terminalia chebula*; h, *Tinospora cordifolia*; i, *Tribulus terrestris*.

K. pneumoniae, *P. mirabilis*, *P. vulgaris* and *P. aeruginosa* were used in this study. All these bacteria were directly isolated from urine samples of UTI patients attending and other patients admitted in the hospital (see, Mishra et al., 2013). Identification of pathogenic bacterial strains was done depending upon gross colony morphology and biochemical tests of isolated pure bacterial cultures, along with Microbial Type Culture Collection (MTCC), Chandigarh, reference strains (Mishra and Padhy, 2013). All bacterial strains were subjected to antibiotic sensitivity tests by the Kirby-Bauer's disc diffusion method, using a 4 mm thick Mueller–Hinton (MH) agar (HiMedia, Mumbai) medium, following the standard antibiotic susceptibility test chart of Clinical Laboratory Standard Institute guidelines (CLSI, 2011). The use of urine samples does warrant ethical approval of the institute.

2.3. Antibacterial test of plant extracts

One strain from each bacterial species having resistance to a maximum number of presently used antibiotics was further used for monitoring antibacterial potentiality of methanol leaf extracts with gentamicin 30 μg/ml as the reference standard, by the agar-well diffusion method, as previously detailed (Mishra and Padhy, 2013; Rath and Padhy, 2014). Antibacterial activities were evaluated as before (Mishra and Padhy, 2013) and results of the third repetition are presented.

2.4. Determinations of MIC and MBC of plant extracts

Original stock solutions of leaf extracts were prepared with methanol, at 44.44 mg plant extract/ml 10% DMSO solution, with distilled water. Each stock solution was diluted to obtain final concentrations of 0.29, 0.67, 1.51, 3.41, 4.27, 9.63, 21.67 and 44.44 mg/ml with the DMSO solution. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanol leaf extracts were determined in a well on a 96-welled (12 × 8) micro-titre plate (Fig. 2), as described elsewhere (Mishra and Padhy, 2013; Rath and Padhy, 2014).

2.5. Qualitative phytochemical analyses

The following tests were performed for selected 9 medicinal plants for the presence of alkaloids, carbohydrates, saponins, flavonoids, steroids/terpenes, tannins, resins, glycosides, and anthraquinones, as detailed previously (Dubey and Padhy, 2013).

2.6. Statistical analysis

Kruskal–Wallis *H* test for data of zone of inhibition as antibacterial activities in agar-cup method of 3 comparable

Table 1 Ethnomedicinal report of 9 medicinal plants used against urinary tract infection causing bacteria.

Sl. No	Plant name	Family	English name; *Local name*	Parts used	Ethnomedicinal uses
1	*Anogeissus acuminata* (Roxb.ex.DC) wall.ex Guill. and perr.)	Combretaceae	Button tree; *Phasi*	Leaf/ Bark	Its leaf has wound healing activity; used for inflammation, urinary tract infection (UTI) and skin diseases. Its bark is used to treat diabetes
2	*Azadirachta indica* L. Adelb	Meliaceae	Neem; *Neemba*	Leaf	It is used as an antiseptic and for antiviral action (chicken pox). It is used for the treatment of acne
3	*Bauhinia variegata* L.	Fabaceae	Mountain ebony; *Kanchan*	Leaf/ Root	Its leaf is used for burning sensation during urination. The roots are used for digestive problems, diarrhoea and throat infections
4	*Boerhaavia diffusa* L. nom. cons.	Nyctaginaceae	Hog weed; *Atika podi*	Leaf	It is used to improve eyesight, to treat UTI, dysentery and diabetes
5	*Punica granatum* L.	Lythraceae	Pomegranate; *Dalimba*	Leaf/ Bark/ Fruits	It is used for diarrhoea, dysentery, intestinal parasites, kidney problems, heart and throat problems; it is used to stop nose bleeds and gum bleeds and as an eye drop to slow the development of cataracts
6	*Soymida febrifuga* Roxb.	Meliaceae	Indian redwood; *Rohini*	Leaf/ Bark	It is used in the treatment of diarrhoea, dysentery, UTI, fever, vaginal infections, rheumatism swellings
7	*Terminalia chebula* Retz.	Combretaceae	Chebulic myrobala; *Harida*	Leaf/ Fruits	Its leaves are used in skin disorders, anaemia, narcosis, piles, fever, diarrhoea, dysentery, cough and UTI; fruits are used for constipation and anorexia
8	*Tinospora cordifolia* (Thunb.) Miers	Menispermaceae	Heart-leaved moonseed; *Guluchi*	Leaf/ Bark	It has hepato-protective activity; commonly it is used for diabetes and also to treat tuberculosis
9	*Tribulus terrestris* L.	Zygophyllaceae	Puncture vine; *Gokhura*	Leaf	It is used to treat kidney, bladder, UTI and sexual problems

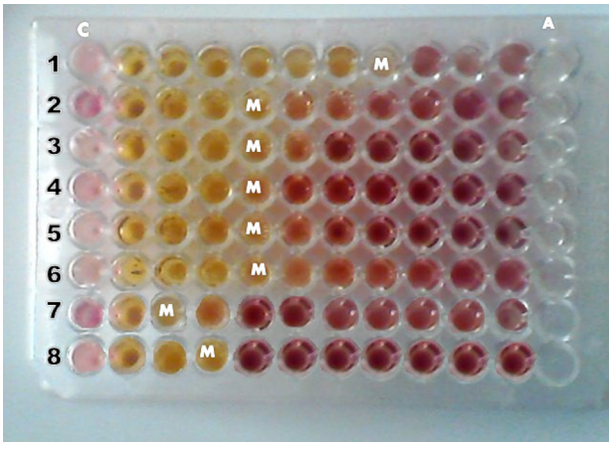


Figure 2 Determination of minimum inhibitory concentrations (MICs), in a 96-well microtiter plate, of 44.44 mg/ml of methanol leaf extract of *P. granatum* against 8 MDR UTI causing pathogenic bacteria (1 = *S. aureus*, 2 = *E. faecalis*, 3 = *A. baumannii*, 4 = *E. aerogenes*, 5 = *K. pneumoniae*, 6 = *K. oxytoca*, 7 = *P. mirabilis*, 8 = *P. vulgaris*). M = MIC at numbers that signifies the lowest concentration of leaf extract. C = control without plant leaf extract; A = Gentamicin (30 μg/ml) as control without any plant leaf extract.

plants against 11 bacteria was done using the Statistical Package for Medical Science version 17.0 (SPSS Inc., IL, USA).

3. Results

3.1. Collection of plants

Ethnomedicinal information on 9 selected medicinal plants are documented along with the details of modalities on crude extracts as medicine for many ailments used by local ethnic aborigine groups (Table 1). Most of these plants are used for infectious diseases and were found edible as medicines by the aborigine society.

3.2. Antibiotic sensitivity test of bacteria

The antibiotic profile of each pathogenic bacterium was determined using specified antibiotic discs (Table 2). GP isolates, *E. faecalis* were resistant to 17 and *S. aureus* were resistant to 13 of 18 antibiotics used. Among the nine GN isolates, *A. baumannii*, *E. aerogenes*, and *E. coli* were resistant to 11, *C. freundii*, *K. pneumoniae*, *K. oxytoca* and *P. aeruginosa* were resistant to 12, *P. mirabilis* and *P. vulgaris* were resistant to 10 antibiotics of the total 14 antibiotics used. The details of individual antibiotics resistant profiles of individual bacteria are presented (Table 2). Thus, all isolated bacterial strains were MDR.

3.3. Antibacterial test of plant extracts

Methanol extracts of medicinal plants when tested against MDR strains of 11 bacteria, 3 plants, *A. acuminata*, *P. granatum* and *S. febrifuga* were seen most effective, with at least causing 25 to 29 mm diameter-sizes of zone of inhibition

Table 2 Antibiotic susceptibility results of multidrug resistant Gram-positive and Gram-negative bacteria.

Bacterium	Susceptibility to prescribed antibiotics																	
	Aminoglycosides		β-lactams				Cephalosporin		Fluoroquinolones				Glyco-peptides		Lincosa-mide	Sulfonamide	Stand alones	
	Ac	Ge	Ak	Am	Ox	Pt	Ce	Cf	Of	Le	Nx	Gt	Tei	Va	Cd	Cot	Ch	Lz
*E. faecalis**	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	R
*S. aureus**	R	R	R	R	MS	R	R	R	R	R	R	R	MS	MS	MS	R	R	S
A. baumannii	R	R	R	R	ND	R	R	R	R	R	MS	S	ND	ND	ND	R	R	S
C. freundii	R	R	R	R	ND	R	R	R	R	R	R	MS	ND	ND	ND	R	R	S
E. aerogenes	R	R	R	R	ND	R	R	R	R	R	R	MS	ND	ND	ND	R	MS	S
E. coli	R	R	R	R	ND	S	R	R	R	R	R	R	ND	ND	ND	S	R	S
K. oxytoca	R	R	R	R	ND	R	R	R	R	R	R	MS	ND	ND	ND	S	R	R
K. pneumoniae	R	R	R	R	ND	R	R	R	R	R	R	S	ND	ND	ND	R	R	S
P. mirabilis	R	R	R	R	ND	S	R	R	S	R	S	MS	ND	ND	ND	R	R	R
P. vulgaris	R	R	R	S	ND	R	R	S	S	R	R	S	ND	ND	ND	R	R	R
P. aeruginosa	R	R	R	R	ND	R	R	R	R	R	R	MS	ND	ND	ND	R	R	S

Note: '*' marked bacteria are Gram-positives and the rest are Gram-negatives. R: Resistant; S: Sensitive; MS: moderately sensitive; ND: not done. Antibiotics (μg/disc), Ac: amikacin 30; Ak: amoxyclav 30; Am: ampicillin 10; Cd: clindamycin 2; Cf: cefpodoxime 10; Ch: chloramphenicol 30; Cot: co-trimoxazole 25; Ce: ceftriaxone 30; Ge: gentamicin 10; Gt: gatifloxacin 5; Nx: norfloxacin 10; Le: levofloxacin 5; Lz: linezolid 30; Of: ofloxacin 5; Ox: oxacillin 1; Pt: piperacillin/tazobactam 100/10; Tei: teicoplanin 5; Va: vancomycin 30.

against any bacterium (Table 3). The *A. acuminata* leaf extract registered the highest value of inhibition zone of 27 mm, against *S. aureus* and the lowest value of 20 mm against *P. mirabilis* was recorded; but, inhibition zone values due to *A. acuminata* were recorded for other given bacteria (mm): *E. faecalis* (24), *A. baumannii* (23), *C. freundii* (22), *E. aerogenes* (21), *E. coli* (22), *K. oxytoca* (23), *K. pneumoniae* (24), *P. vulgaris* (21) and *P. aeruginosa* (25). Thus, *A. acuminata* extract was effectively capable of controlling all the 11 MDR bacteria. Methanol leaf extract of *A. indica* had shown the highest inhibition-zone size of 22 mm against *E. aerogenes*, while the lowest value was 12 mm against *P. mirabilis*. Methanol leaf extract of *P. granatum* had the highest value of inhibition-zone size, 26 mm against *S. aureus* and the lowest value of 17 mm was against *P. mirabilis*; the extract was effectively capable of controlling all the 11 MDR pathogens by registering values of inhibition zones ranging from 18 to 25 mm. The highest value of inhibition-zone size of 25 mm against *S. aureus* and the lowest value of 17 mm against *E. aerogenes* had been noted due to the methanol extract of *S. febrifuga*; and 20, 23, 21, 19, 22, 21, 19, 20 and 28 mm values of size of zone of inhibition were recorded against the pathogenic bacteria, *E. faecalis*, *A. baumannii*, *C. freundii*, *E. coli*, *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *P. aeruginosa*, respectively. The methanol leaf extract of *S. febrifuga* had an effective controlling capacity over all the pathogens. Methanol leaf extracts of the rest 6 plants had moderate control capacity on all bacterial strains (Table 3). The total size of zone of inhibition of all used plants is arranged according to the decreasing order, *A. acuminata* > *P. granatum* > *S. febrifuga* > *A. indica* > *B. variegata* > *T. terrestris* > *T. cordifolia* > *T. chebula* > *B. diffusa*. Moreover, Kruskal–Wallis *H* test for data of zone of inhibition of the 3 best plants, *A. acuminata*, *P. granatum* and *S. febrifuga* yielded the *H*-value of 0.83, which was statistically not significant; so, these 3 plants were inferred as equally effective for antibacterial activity.

3.4. MIC and MBC of plant extracts

The methanol leaf extract of *A. acuminata* had the lowest MIC value, 0.29 mg/ml and the lowest MBC value 0.67 mg/ml against *S. aureus*; MIC value of 0.67 mg/ml and MBC value of 1.51 mg/ml against *E. faecalis*, *K. pneumoniae* and *P. aeruginosa*, while MIC value of 1.51 mg/ml and MBC value of 3.41 mg/ml against *A. baumannii* and *K. oxytoca* were recorded *by A. acuminata*. On the other hand, the highest MIC value of 3.41 mg/ml, and the highest MBC value of 4.27 mg/ml due to *A. acuminata* extract were noted for *C. freundii*, *E. aerogenes*, *E. coli*, *P. mirabilis* and *P. vulgaris*. The methanol leaf extract of *P. granatum* showed the lowest MIC value of 0.29 mg/ml and the lowest MBC value of 0.67 mg/ml against *S. aureus*; MIC value of 0.67 mg/ml and MBC value of 1.51 mg/ml was noted against *C. freundii*, *E. coli* and *P. aeruginosa*; MIC value of 1.51 mg/ml and MBC value of 3.41 mg/ml was recorded against *E. faecalis*; MIC value of 3.41 mg/ml and MBC value of 4.27 mg/ml were recorded against *A. baumannii*, *E. aerogenes*, *K. oxytoca* and *K. pneumoniae*. The highest MIC value of 4.27 mg/ml and MBC value of 9.63 mg/ml by the extract of *P. granatum* were noted against *P. mirabilis* and *P. vulgaris*. The methanol leaf extract of *S. febrifuga* showed the lowest MIC value of 0.67 mg/ml and the lowest MBC value of 1.51 mg/ml against *S. aureus*. MIC value of 1.51 mg/ml and MBC value of 3.41 mg/ml were recorded against *A. baumannii*. By the extract of *S. febrifuga*, MIC value 3.41 mg/ml and MBC value 4.27 mg/ml were noted against *E. faecalis*, *C. freundii*, *K. oxytoca*, *K. pneumoniae* and *P. vulgaris* and the highest MIC value of 4.27 mg/ml and MBC value of 9.63 mg/ml were recorded against *E. aerogenes*, *E. coli*, *P. mirabilis* and *P. aeruginosa*. The MIC and MBC values of leaf extracts of rest other plants were recorded (Table 4). A lower MIC/MBC value signifies that a minimum amount of plant extract is used, whereas, a higher value signifies the use of comparatively more amount of plant extract for the control of any bacterium.

Table 3 Antibacterial activity as size of zone of inhibition due to 9 selected medicinal plants against bacteria with gentamicin 30 μg/ml as the positive control.

Bacteria	Size of zone of inhibition by plants (Nos. 1 to 9) methanol extracts (mm)									
	A. acuminata	*A. indica*	*B. variegata*	*B. diffusa*	*P. granatum*	*S. febrifuga*	*T. chebula*	*T. cordifolia*	*T. terrestris*	Ge 30 μg/ml
E. faecalis	24	18	17	15	23	20	18	17	19	25
S. aureus	27	20	21	17	26	25	23	19	21	28
A. baumannii	23	17	14	13	22	23	15	–	15	20
C. freundii	22	21	13	12	24	21	–	13	13	21
E. aerogenes	21	22	15	14	20	17	–	14	–	23
E. coli	22	19	19	10	25	19	14	16	17	26
K. oxytoca	23	15	15	13	21	22	15	15	16	22
K. pneumoniae	24	17	17	15	18	21	13	13	15	20
P. mirabilis	20	12	12	–	17	19	11	11	18	22
P. vulgaris	21	13	14	–	19	20	–	–	17	23
P. aeruginosa	25	15	20	15	24	18	16	18	20	26
Total zone size	252	189	177	124	239	225	125	136	171	

Note: Numbers 1 to 9 are serial numbers of plants given in Table 1; Ge, gentamicin. Values are measurements of zone of inhibition due to methanol-extracts. "–" sign denotes no activity. Kruskal–Wallis *H* test for data of zone of inhibition of 3 plants, *A. acuminata*, *P. granatum* and *S. febrifuga* yielded the *H*-value of 0.83, which was statistically not significant; so, these 3 plants were equally effective for antimicrobial activity. The rest other 6 plants were clearly lesser in antimicrobial activity in comparison to cited 3 plants. It was confirmed that 10% DMSO had no inhibitory effect on any bacterium.

3.5. Qualitative phytochemical analyses

Qualitative phytochemical analyses were done for methanol leaf extracts. Phytochemicals, alkaloids, flavonoids, carbohydrates, terpenoids, steroids, tannins, resins, saponins and anthraquinones, which could be attributed to the recorded significant antibacterial activities in most extracts (Table 5). From PubChem database, structure and related information of one leading antimicrobial compound of each plant was presented (Table 6). From previous studies, the antibacterial nature of these cited 9 compounds were ascertained (Table 6). The Molsoft tool (http://molsoft.com/mprop/) was used to find out the drug likeness scores of each compound, according to their structure canonical 'simplified molecular-input line-entry system' (SMILES). Thus theoretically, based on the available data on drug likeness scores, phytochemicals could be arranged in the decreasing order of drug-likeness scores mentioned against: quercetin (0.93) > berberin (0.91) > luteolin-7-O-glugoside (0.86) > kaempferol (0.77) > ursolic acid (0.65) > argungenin (0.61) and mahmoodin (0.61) > conocarpan (0.13) and dihydrodedrodehydrodiconiferylalcohol (0.13) > anolignan B (−0.78). It could be inferred here that *S. febrifuga* among the 3 best plants is the most leading plant with luteolin-7-O-glugoside, which has a good score of drug-likeness (Table 6).

4. Discussion

All of these 9 plants were used by an ethnic tribe, the Kandha tribe of Kalahandi district, since time immemorial for primary healthcare needs specifically for infectious diseases. It was seen that, 11 bacteria isolated from urine samples were resistant to the following: aminoglycosides, β-lactams (amoxyclav and ampicillin), two cephalosporins (ceftriaxone and cefpodoxime) as well as, chloramphenicol, signifying most bacterial strains as resistant to most antibiotics. Additionally, plants, *A. acuminata*, *A. indica*, *B. variegata*, *P. granatum* and *S. febrifuga* had control capacity on all the 11 strains of MDR bacteria. Moreover, among these 3 best plants in the control of MDR bacteria *in vitro* were *A. acuminata*, *P. granatum* and *S. febrifuga*. *P. granatum* has stigmasterol, a sterol with the drug-likeness score, 0.73 while, *S. febrifuga* has luteolin-7-O-glucoside, a flavonoid with the drug-likeness score, 0.86. Further work on antibacterial bioactive compounds of *A. acuminata* is in progress, as it had significant microbial activity. However, the plant, *T. terrestris* has the famous antimicrobial agent, quercetin, which has an effective drug likeness score of 0.93; but this plant did not have the best antibacterial activity, in this study or with these bacteria.

Antibiotic sensitive pathogens have a limited capacity of virulence as the employed antibiotic controls them *in vivo*. At a particular density, the host defense system too helps control pathogens, when the later are in a limiting number. As known, for the internal protection, antibiotic producing organisms harbour antibiotic resistant genes in plasmids and chromosomes, as well as the transfer mechanisms remain active (Mamelli et al., 2009). Therefore, such genes and/or transposon are taken up, horizontally by the susceptible group of bacteria, through bacterial transformation and/or conjugation (Pages et al., 2008; Warnes et al., 2012).

Moreover, bacteria having simple genomes undergo intrinsic (mutations) or acquired genetic (conjugations and transformation) changes in the presence of an antibiotic, as a stress factor (Groisman and Ochman, 1996). As a result, accrual antibiotic resistance mechanisms are the clinical determinants of the pathogenesis. It had been known that in areas, where a particular group of antibiotics are used bacteria resistance to same antibiotics were in higher numbers (Shrestha et al., 2002). 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' (Groisman and Ochman, 1996). Progressively, the use of more antibiotics even of higher generations for the control of infectious diseases have led to multiple resistances, i.e., too many antibiotics are ineffective to progressively increasing

Table 4 MIC and MBC values of selected 9 medicinal plants and of gentamicin as the positive control against bacteria.

Bacterium	MIC and MBC values by methanol extracts of 9 plants (mg/ml)																		Gentamicin μg/ml	
	1		2		3		4		5		6		7		8		9			
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	0.67	1.51	3.41	4.27	4.27	9.63	9.63	21.67	1.51	3.41	3.41	4.27	4.27	9.63	4.27	9.63	4.27	9.63	0.93	1.87
S. aureus	0.29	0.67	3.41	4.27	3.41	4.27	4.27	9.63	0.29	0.67	0.67	1.51	1.51	3.41	4.27	9.63	3.41	4.27	0.46	0.93
A. baumannii	1.51	3.41	4.27	9.63	9.63	21.67	9.63	21.67	3.41	4.27	1.51	3.41	9.63	21.67	–	–	9.63	21.67	3.75	7.50
C. freundii	3.41	4.27	3.41	4.27	9.63	21.67	9.63	21.67	0.67	1.51	3.41	4.27	–	–	9.63	21.67	9.63	21.67	1.87	3.75
E. aerogenes	3.41	4.27	3.41	4.27	9.63	21.67	9.63	21.67	3.41	4.27	4.27	9.63	–	–	9.63	21.67	–	–	0.93	1.87
E. coli	3.41	4.27	4.27	9.63	4.27	9.63	–	–	0.67	1.51	4.27	9.63	9.63	21.67	4.27	9.63	4.27	9.63	1.87	3.75
K. oxytoca	1.51	3.41	9.63	21.67	9.63	21.67	9.63	21.67	3.41	4.27	3.41	4.27	9.63	21.67	9.63	21.67	4.27	9.63	0.93	1.87
K. pneumoniae	0.67	1.51	4.27	9.63	4.27	9.63	9.63	21.67	3.41	4.27	3.41	4.27	9.63	21.67	9.63	21.67	9.63	21.67	1.87	3.75
P. mirabilis	3.41	4.27	9.63	21.67	9.63	21.67	–	–	4.27	9.63	4.27	9.63	9.63	21.67	9.63	21.67	4.27	9.63	3.75	7.50
P. vulgaris	3.41	4.27	9.63	21.67	9.63	21.67	–	–	4.27	9.63	3.41	4.27	–	–	–	–	4.27	9.63	1.87	3.75
P. aeruginosa	0.67	1.51	9.63	21.67	3.41	4.27	9.63	21.67	0.67	1.51	4.27	9.63	9.63	21.67	4.27	9.63	3.41	4.27	0.46	0.93

Note: Numbers 1 to 9 are serial numbers of plants given in Table 1. Values are measurements of MIC and MBC due to methanol extracts. "–" sign denotes no activity; Gentamicin was used as dilutions from 30 μg/ml.

Table 5 Qualitative phytochemical analyses of methanol extracts of 9 medicinal plants.

Sl.No.	Plants	Alkaloids	Resins	Glycosides	Terpenoids	Carbohydrates	Saponins	Tannins	Flavonoids	Steroids	Anthraquinones
1	*A. acuminata*	+	–	+	+	+	+	+	+	+	+
2	*A. indica*	–	+	+	+	+	+	–	–	+	–
3	*B. variegata*	+	+	+	–	+	+	+	–	+	+
4	*B. diffusa*	+	–	–	–	–	–	+	+	+	+
5	*P. granatum*	+	–	+	+	–	+	+	+	+	+
6	*S. febrifuga*	+	+	+	+	+	–	+	+	+	+
7	*T. chebula*	+	+	+	+	+	–	+	+	–	+
8	*T. cordifolia*	+	–	+	–	+	+	–	–	+	–
9	*T. terrestris*	–	–	–	+	–	–	–	–	+	–

Note: "+" sign denotes presence, and "– "sign denotes absence of the compound in a plant.

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 bacterial resistance. Older antibiotics slowly become moribund, 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 antimicrobial would be present in parallel along with the employed antibiotic, there would be the coveted blithesome result, since no bacterium how much genetically well-equipped be it may as in a cohort of MDR bacteria, can never over-ride complexities of phytochemicals for survival. This fact is repeatedly seen *in vitro* with several plant extracts (Dubey and Padhy, 2013; Mishra and Padhy, 2013; Rath and Padhy, 2013; Sahu et al., 2015). Thus, those in a coalesced manner, as in a crude extract, have a combined controlling effect.

It has been demonstrated with *Salmonella enterica* serotype *typhimurium* (Alekshun and Levy, 1999). Moreover, MDR *Neisseria gonorrheae* had been known to acquire '*MTR and SAP A MDR'* systems of genes, from *S. enterica* serotype *typhimurium* (Hagman and Shaferm, 1995). Discovery and development of antibiotics in the last century have not only saved countless human lives, but have provided assurances in clinical management all over. But, concomitant development of antibiotic-resistance mainly in bacteria has dismayed both preventive and therapeutic potencies of antibiotics today. In the odyssey of drug development, antibiotics are introduced continually and a few of them are modified suiting to the need to overcome bacterial resistance. Eventually, today there are a large number of antibiotics in use. The demand for newer antibiotics for MDR bacteria in colossal scale has arisen, which has become difficult to meet, as these small molecules are extremely complex in functionality linked to chemical structure. Secondly, an antibiotic ensconced for a typical set of infections cannot ordinarily be abandoned as an obsolete drug, due to reports of dogmatic/realistic resistance in a geographical zone; rather, along with the same antibiotic the introduction of complementary or adjuvant drug could be aimed, when considered with contemplation the problem of morbidity/mortality from infections due to MDR bacteria (Davies and Davies, 2010).

Applied antibiotics, being of microbial origin, are readily won over by pathogenic microbes *in vivo*. The cell producing an antibiotic has the characters of the self-protective mechanism as characters/genes, which direct the modes of resistance such as, alteration in the cell membrane by efflux mechanism or production of external enzymes like, β-Lactamases are intrinsically transmitted to similar bacteria, as discussed (Rout et al., 2014). In short, suitable antibiotics are required in colossal scale globally, which would give a way to the chance of development of resistant strain(s) of pathogenic bacteria in a Darwinian way, further. Indeed, the present methodology of methanol-extraction of phytochemicals is a unique approach, as this solvent helps extraction of most polar to non-polar phytocompounds (Rezaie et al., 2015). The β-Lactam group of antibiotics consisting of penicillins, cephalosporins, monobactams, glycopeptides and penems target the peptidoglycan biosynthesis of bacteria. Tetracyclines, aminoglycosides, macrolides, lincosamides, streptogramins, oxazolidinone (linezolid) and phenicols cause a thwart to the translation process in the parasitic cell; quinolones inhibit the DNA replication. Pyrimidines and sulphonamides alter carbon metabolism during inhibition of parasitic growth; and the most recent ones such as, daptomycin and colistin inhibit cell membrane functions (see, Davies and Davies, 2010). However, the first effective antimicrobials were the sulphonamides, which have been amply lent themselves for further uses in the drug development process as antibacterials in the last several decades. Thus, the concept of complementary use of phytocompounds along with main stream drugs, the antibiotics has immersed when the myriad mechanisms of drug resistance is considered in the face of success of phytocompounds as non-microbial antimicrobials (Sahu et al., 2015).

Our school has screened out about 250 plants in the last 4 years (Rath and Padhy, 2012; Rath et al., 2012; Dubey and Padhy, 2012; Mishra and Padhy, 2013; Rath and Padhy, 2014; Sahu et al., 2015), using mainly ethanol and water as extracting solvents. Among them for 47 plants methanol was used as the solvent (Mishra and Padhy, 2013; Rath and Padhy, 2014). A comparative account of MIC values of the best plants among 47 against MDR strains of 8 gruesome

Table 6 Leading antimicrobial phytochemicals structure, information with properties.

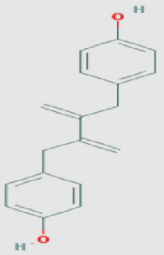
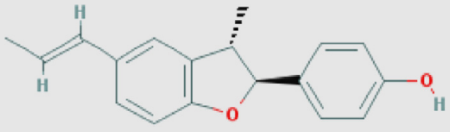
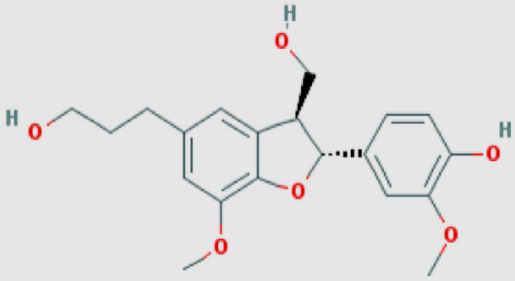
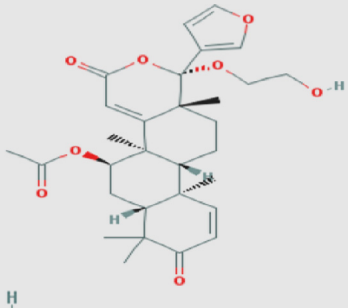
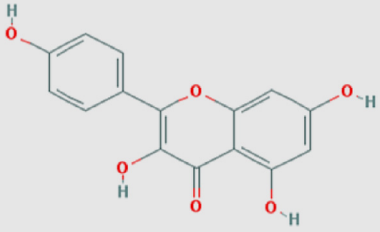
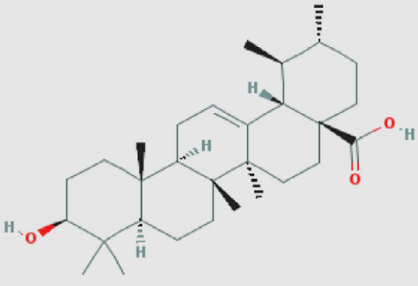
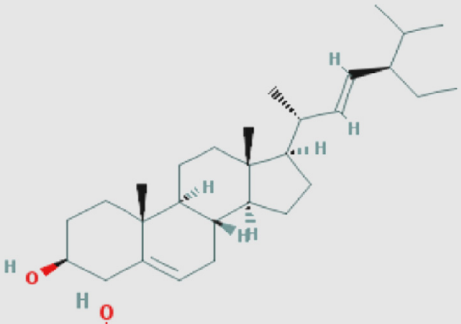
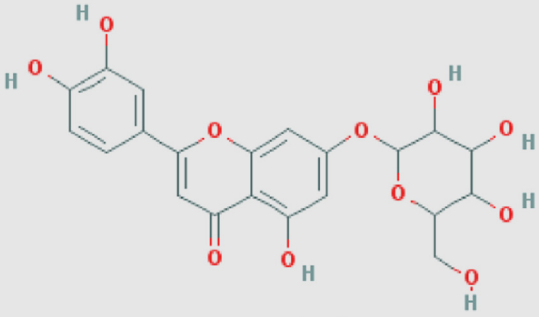
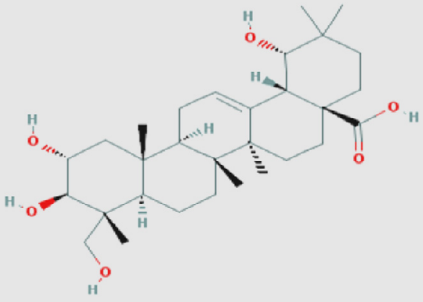
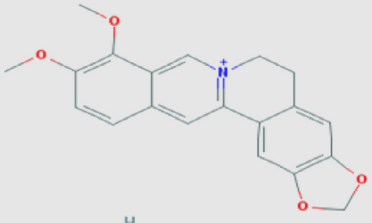
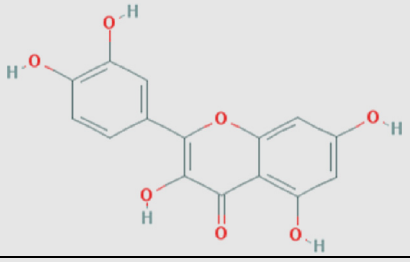
Plant name	Leading antimicrobial phytochemical structure	Information and properties of leading phytochemical	Drug-likeness score	References
A. acuminata		Anolignan B (oc) Molecular weight: 266.33432 [g/mol] Molecular formula: $C_{18}H_{18}O_2$ XLogP3-AA: 5.5 H-Bond donor: 2 H-Bond acceptor: 2	−0.78	
		Conocarpan (oc) Compound ID: 6474521 Molecular Weight: 266.33432 [g/mol] Molecular Formula: $C_{18}H_{18}O_2$ XLogP3-AA: 4.4 H-Bond donor: 1 H-Bond acceptor: 2	0.13	Rimando et al. (1994a,b), Eldeen et al. (2006)
		Dihydrodehydrodiconiferylalcohol (oc) Compound ID: 5274623 Molecular weight: 360.40096 [g/mol] Molecular formula: $C_{20}H_{24}O_6$ XLogP3-AA: 2.1 H-Bond donor: 3 H-Bond acceptor: 6	0.13	
A. indica		Mahmoodin (l) Compound ID: 126566 Molecular weight: 526.61792 [g/mol] Molecular formula: $C_{30}H_{38}O_8$ XLogP3-AA: 3.7 H-Bond donor: 1 H-Bond acceptor: 8	0.61	Siddiqui et al. (1992)
B. variegata		Kaempferol (f) Compound ID: 5280863 Molecular weight: 286.2363 [g/mol] Molecular formula: $C_{15}H_{10}O_6$ XLogP3: 1.9 H-Bond donor: 4 H-Bond acceptor: 6	0.77	Holler et al. (2012)
B. diffusa		Ursolic acid (t) Compound ID: 64945 Molecular weight: 456.70032 [g/mol] Molecular formula: $C_{30}H_{48}O_3$ XLogP3-AA: 7.3 H-Bond donor: 2 H-Bond acceptor: 3	0.65	Jiménez-Arellanes et al. (2013)

Table 6 (*continued*)

Plant name	Leading antimicrobial phytochemical structure	Information and properties of leading phytochemical	Drug-likeness score	References
P. granatum		Stigmasterol (s) Compound ID: 5280794 Molecular weight: 412.69082 [g/mol] Molecular formula: $C_{29}H_{48}O$ XLogP3-AA: 8.6 H-Bond donor: 1 H-Bond acceptor: 1	0.73	Awouafack et al. (2013)
S. febrifuga		Luteolin-7-O-glucoside (f) Compound ID: 5291488 Molecular weight: 448.3769 [g/mol] Molecular formula: $C_{21}H_{20}O_{11}$ XLogP3-AA: 0.5 H-Bond donor: 7 H-Bond acceptor: 11	0.86	Khatkar et al. (2014)
T. chebula		Arjungenin (t) Compound ID: 12444386 Molecular weight: 504.69852 [g/mol] Molecular formula: $C_{30}H_{48}O_6$ XLogP3-AA: 4.5 H-Bond donor: 5 H-Bond acceptor: 6	0.61	Manosroi et al. (2013)
T. cordifolia		Berberin (a) Compound ID: 2353 Molecular weight: 336.36122 [g/mol] Molecular formula: $C_{20}H_{18}NO_4^+$ XLogP3-AA: 3.6 H-Bond donor: 0 H-Bond acceptor: 4	0.91	Choudhary et al. (2013)
T. terrestris		Quercetin (f) Compound ID: 5280343 Molecular weight: 302.2357 [g/mol] Molecular formula: $C_{15}H_{10}O_7$ XLogP3: 1.5 H-Bond donor: 5 H-Bond acceptor: 7	0.93	Rashed and Butnariu (2014)

Note: a, alkaloid; f, flavonoid; l, limonoid; oc, organic compound; s, sterol; t, terpene.

bacteria is considered, along with the present 3 best plants (Table 7). It is discernible that methanol extracts of most plants had MIC values as 3.41 or more, except those of *Cinnamomum tamala*, *A. acuminata*, *P. granatum* and *S. febrifuga*, which had comparatively lower MIC values, as 1.51 or 0.67 or 0.29 mg/ml.

Table 7 MIC values (mg/ml) of methanol leaf-extracts of plants against pathogenic bacteria.

Bacteria Plants	*Ef*	*Sa*	*Ab*	*Cf*	*Ea*	*Ec*	*Kp*	*Pa*	References
Allium sativum	NE	9.63	9.63	4.27	3.41	NE	9.63	NE	Rath and Padhy (2014)
Amomum aromaticum	3.41	4.27	NE	NE	9.63	NE	NE	4.27	Rath and Padhy (2014)
Artocarpus heterophyllus	9.63	9.63	9.63	9.63	9.63	NE	9.63	9.63	Mishra and Padhy (2013)
Cinnamomum tamala	3.41	1.51	NE	NE	3.41	NE	9.63	4.27	Rath and Padhy (2014)
Dalbergia latifolia	9.63	3.41	9.63	9.63	9.63	9.63	NE	4.27	Mishra and Padhy (2013)
Gmelina arborea	9.63	3.41	9.63	9.63	NE	9.63	9.63	9.63	Mishra and Padhy (2013)
Melia azedarach	9.63	3.41	9.63	NE	NE	NE	NE	9.63	Mishra and Padhy (2013)
Mentha spicata	NE	9.63	9.63	NE	1.51	NE	3.41	4.27	Rath and Padhy (2014)
Mimusops elengi	4.27	4.27	9.63	NE	9.63	9.63	9.63	9.63	Mishra and Padhy (2013)
Myristica fragrans	3.41	NE	NE	4.27	3.41	NE	9.63	9.63	Rath and Padhy (2014)
Pongamia pinnata	9.63	4.27	NE	NE	9.63	NE	NE	9.63	Mishra and Padhy (2013)
Pterocarpus marsupium	4.27	4.27	9.63	9.63	9.63	9.63	NE	9.63	Mishra and Padhy (2013)
Shorea robusta	9.63	4.27	9.63	9.63	NE	NE	9.63	3.41	Mishra and Padhy (2013)
Anogeissus acuminata	0.67	0.29	1.51	3.41	3.41	3.41	0.67	0.67	Present work
Punica granatum	1.51	0.29	3.41	0.67	3.41	0.67	3.41	0.67	Present work
Soymida febrifuga	3.41	0.67	1.51	3.41	4.27	4.27	3.41	4.27	Present work

Note: Ef, *E. faecalis*; *Sa*, *S. aureus*; *Ab*, *A. baumannii*; *Cf*, *C. freundii*; *Ea*, *E. aerogenes; Ec*, *E. coli*; *Kp*, *K. pneumoniae*; *Pa*, *P. aeruginosa*, ND, not done; NE, no effect.

5. Conclusion

Antibiograms of 11 isolated pathogenic bacteria with 17 antibiotics of the day ascertained that all were amply MDR. The work on individual 9 plants in controlling all MDR strains of bacteria was evident, mostly with lower MIC and MBC values. All these used plants have ethnomedicinal uses and 3 best plants could be promoted as complementary medicine. The recorded data of 3 best plants, *A. acuminata*, *P. granatum* and *S. febrifuga*, are anticipated to trigger work on the isolation of pure compounds for further scientific use in the crusade of the control of MDR bacteria. Phytocompounds, stigmasterol and luteolin-7-O-glucoside already isolated from the second and the third best antibacterial plant, respectively have significant drug-likeness scores. Thus, the presently used three best plants could be regarded as the most effective plants studied for further consideration for complementary medicine as sources of non-microbial anti-microbials against most MDR UTI causing bacteria.

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