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Experimental Article

In vitro antibacterial efficacy of plants used by an Indian aboriginal tribe against pathogenic bacteria isolated from clinical samples

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الملخص

أهداف البحث: تقييم فعاليات ٢١ نباتا طبيا تستخدمه قبيلة هندية من السكان الأصليين في علاج أمراض معدية تسببها بكتيريا تم عزلها من عينات سريرية.

طرق البحث: تم استخدام الأساليب الكيميائية الحيوية المتعارف عليها للتعرف على البكتيريا المستخلصة من عدة عينات سريرية. وعُرضت جميع سلالات البكتيريا لاختبارات التحسس بطريقة "كربي - باير" للانتشار القُرصي. وتبين من نتائج تخطيط المضادات للبكتيريا إيجابية الصبغة والبكتيريا سلبية الصبغة المعزولة وجود عينات مقاومة لعدة مضادات حيوية. تم تعريض المستخلص الميثانولي لورقة نبات سولانم زانثوكاربم للتحليل الضوئي عن طريق الفصل اللوني الرقيق. أجريت الترسية الجزيئية لإنزيم بيتا-لاكتميز من بكتيريا استرتشيا كولاي مع العناصر الضوئية لسولانم زانثوكاربم لتحديدالمادة الفاعلة.

النتائج: أكثر خمس نباتات فاعلية ضد البكتيريا المقاومة لعدة مضادات حيوية وتسببت في مناطق تثبيط من ٢١-٢٧ مم كانت بوتشانانية لاتيفوليا، وكاريا اربوريا، وأوسيمم تنيوفلورم، وسينا ألاتا، وسولانم زانثوكاربم. تميزت سولانم زانثوكاربم بكل من: أقل قيمة لأدنى تركيز مثبط وكان ٠،٦٧ مجم/مل وأقل قيمة لأدنى تركيز قاتل للبكتيريا وكان ١،٥١ مجم/مل ضد بكتيريا استرتشيا كولاي. عند الدراسة بالفصل اللوني الرقيق سجلت ٩ بقع ميثانول من ورق نبات سولانم زانثوكاربم بنظامي إذابة. كان لكل من جلوكوسيدات الستجماستيرول والسولاسودين اللذين هما الكيميائيات الضوئية لسولانم زانثوكاربم أعلى قيم ترسية ضد إنزيم بيتالاكتميز التي بلغت-١٠،٤٣٩ كيلو كالوري/ مول و-١٠،٨٦٨ كيلو كالوري/ مول على الترتيب.

الاستنتاجات: قد تُثبت هذه الدراسة فاعلية مختبرية لخمس نباتات غير مألوفة ضد بكتيريا مسببة لأمرض ومقاومة لعدة مضادات حيوية. سُجلت كل من جلوكوسيدات الستجماستيرول والسولاسودين حسابيا كأفضل مادتين مستخلصتين من النبتة سولانم زانثوكاربم.

الكلمات المفتاحية: الفاعلية ضد البكتيريا؛ النباتات الطبية؛ سولانم زانثوكاربم؛ الترسية الجزئية؛ بيتالاكتميز

Abstract

Objectives: To evaluate antibacterial efficacies of 21 medicinal plants used by an Indian aboriginal tribe against infectious diseases caused by bacteria isolated from clinical samples.

Methods: Standard biochemical procedures were followed for identifying bacteria that were isolated from several clinical samples. All of the bacterial strains were subjected to antibiotic sensitivity tests by Kirby–Bauer's disc diffusion method. From antibiograms of isolated Gram-positive and Gram-negative bacteria, it was discernible that samples were multidrug resistant (MDR). The methanol leaf-extract of *Solanum xanthocarpum* was subjected to thin layer chromatography (TLC) for phytochemical analysis. Molecular docking of β-lactamase enzyme of *Escherichia coli* with phytochemicals of *S. xanthocarpum* was performed to locate effective compounds.

Results: The most effective 5 plants, which caused the size of the zone of inhibition to range from 21 to 27 mm, were *Buchanania latifolia, Careya arborea, Ocimum tenuiflorum, Senna alata* and *S. xanthocarpum*, for MDR bacteria. *S. xanthocarpum* had the lowest MIC value of 0.67 mg/ml and the lowest MBC value of 1.51 mg/ml against *E. coli*. In the TLC study, 9 spots of methanol leaf-extract of *S. xanthocarpum* were recorded with two solvent systems. The phytochemicals of *S. xanthocarpum*,

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solasodine and stigmasterol glucoside had the highest docking score values, −10.868 kcal/mol and −10.439 kcal/mol, respectively, against β-lactamase.

Conclusion: This study could prove *in vitro* antimicrobial efficacy of 5 uncommon plants against MDR pathogenic bacteria. Solasodine and stigmasterol glucoside were computationally recorded as the best controlling chemicals from the plant *S. xanthocarpum*.

Keywords: Antibacterial efficacy; Medicinal plants; Molecular docking; *Solanum xanthocarpum*; β-lactamase

Introduction

Annoyance from infectious diseases caused by drug/antibiotic resistant bacteria is commonplace in clinical management.[1,2] Oftentimes, newly added antibiotics control infections from resistant bacteria; however, over time, strains that are resistant to the new antibiotics also develop continually.[1] Thus, in the continual process of drug development, several antibiotics, for example penicillin and their derivatives, have been introduced by the structural modification(s) of penicillin.[3] Furthermore, the penicillin group with the β-lactam ring (amoxicillin, ampicillin, azlocillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, methicillin, nafcillin, oxacillin, penicillins, piperacillin, temocillin and ticarcillin) as well as the cephalosporin group of antibiotics have been developed in progressive generations[3]; the cephalosporins had 5 generations, as listed: the first/second generations (cefamandole, cefmetazole, cefonicid, cefotetan, cefoxitin, cefprozil, cefuroxime, cefuzonam), the third generation (cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefodizime, cefotaxime, cefpimizole, cefpodoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime), the fourth generation (cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome) and the fifth generation (ceftobiprole, ceftaroline). Similarly, among other groups, aminoglycosides (e.g., amikacin, gentamicin, amoxyclav, ampicillin), fluoroquinolones (e.g., ciprofloxacin, gatifloxacin, nalidixic acid, norfloxacin, ofloxacin, etc.), glycopeptides (vancomycin and cotrimoxazole) and several stand-alone antibiotics have been introduced, which also have become progressively moribund due to the development of drug resistance.[4] Naturally, antibiotic-resistant bacteria have higher degrees of virulence, compared with the corresponding wild strains, and a multidrug resistant (MDR) bacterium that is resistant to a number of antibiotics is intractable[5] and sometimes precipitates public health episodes, e.g., due to the enteropathogen *Clostridium difficile*.[6]

It should be noted that the horizontal gene transfer of resistance character(s) causes movement to different bacteria of the same, similar or different heritage, so much so that progressively more antibiotic resistant bacteria emerge[7,8]; for example, although it was once clinically known to be a harmless commensal, the Gram-positive (GP) bacterium *Staphylococcus aureus* has now become drug resistant to a group of narrow-spectrum β-lactam antibiotics, the methicillin. The resistant strain, called methicillin-resistant *S. aureus* (MRSA), has become peripatetic and has caused grievous problems in surgical site infections, burn site infections, and urinary tract infections (UTI), which all lead to bacteremia in the lungs, septicaemia and innards, such as persistent lung infection and endocarditic, which cause morbidity and unexpected mortality.[9] The acquired armamentaria by MRSA are resistant to most antibiotics of most other groups, as reported from this hospital, which could be labelled as MDR MRSA[10]; such strains are the super-bug of the health domain. It has been further observed in this hospital that the strains of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have the causatives of major infectious agents in repeated studies of surveillance.[11,12] Even a metallo-β-lactamase strain of *K. pneumoniae* was recorded to cause the mortality of a neonate in this hospital.[13]

Our school has recorded a handful of plants that control MDR strains of pathogenic bacteria, *Enterococcus faecalis, S. aureus* (MRSA), *E. coli*, *K. pneumoniae, P. aeruginosa* and a few more.[14,15] Indeed, regardless of how well a bacterium had picked up resistance to different groups of antibiotics, such a strain was controlled consistently by crude extracts of several plant species.[11,16,17] Continuing this line of work, the antibacterial efficacy of 21 medicinal plants used by an Odishan aborigine group since before recorded history is described here. For the screening work, ten pathogenic bacteria have been isolated from clinical samples of patients who were admitted to the intensive care unit (ICU), wards and cabins of a hospital. The gargantuan numbers of unexplored plants used by aborigines for their health care needs, especially for infectious ailments, require scientific validation as non-microbial antimicrobials for the suitable use of selected lesser known and unknown plants of the forest floor with hill-dwelling tribes, in modern drug development for the crusade against MDR bacteria. A dislogistic approach to the phyto-drugs for their use in mainstream medicine as 'complementary medicine' would be an under-exploitation of medicines from a natural source.

Materials and Methods

Collection of plants and extract preparations

Approximately 60 respondents from 25 hamlets of the Eastern mountain range between Junagarh and Bhawanipatna in the Kalahandi district, Odisha were interviewed in the forest reviewed, and the ethnomedicinal information of 21 plants was documented. The snowball method of survey and sampling was used, as previously followed.[18] Collected leaf samples were crushed to powder form; many 10-g leaf powder samples were dissolved in an aliquot of 100-ml methanol and incubated at 4 °C for 72 h, and the

suspension was filtered. The methanol filtrate was concentrated in a rotary evaporator at 40 °C, until a sticky mass was obtained that was weighed and dissolved in an aliquot of 1.0 ml 10% v/v dimethyl sulfoxide (DMSO), and those were stored at 4 °C until further use.

Isolation, identification of bacterial strains and antibiotic sensitivity test

Two GPs, *E. faecalis*, *S. aureus*, and 8 GNs, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *E. coli*, *K. pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris* and *P. aeruginosa*, were used in this study. All of these bacteria were directly isolated from urine samples of patients from ICUs, wards and cabins at Institute of Medical Sciences and Sum Hospital, Bhubaneswar, using an appropriate medium, which was specific for each bacterium. All of the 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, 2011.[19]

Antibacterial test of plant extracts

One strain from each bacterial species that showed resistance to a maximum number of antibiotics was further used for monitoring the antibacterial potentiality of plant extracts with gentamicin 30 µg/ml, as the standard, by the agar-well diffusion method, as detailed earlier.[17] Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition on a bacterial lawn due to a plant extract; experiments of each solvent extract were conducted three times, and the results of the third repetition are presented. It was confirmed that 10% DMSO had no inhibitory effect on any bacterium.

Determinations of MIC and MBC of plant extracts

Minimum inhibitory concentration (MIC) and *minimum bactericidal concentration* (MBC) of the active plant extracts were determined. Stock solutions of leaf extracts were prepared at 100 mg/ml with 10% DMSO solution. Each stock solution was diluted to obtain the final concentrations of 0.05, 0.13, 0.29, 0.67, 1.51, 3.41, 4.27, 9.63, 21.67 and 44.42 mg/ml. A separate experiment was conducted for each plant-extract. An aliquot of 80 µl of each dilution of a plant-extract was released to a well on a 96-welled (12 × 8) microtitre plate, along with an aliquot of 100-µl MH broth, an aliquot of 20-µl bacterial inocula (10^9 CFU/ml) and a 5-µl aliquot of 0.5% of 2,3,5-triphenyl tetrazolium chloride (TTC). After pouring all of the above into a well, the microplate was incubated at 37 °C for 18 h. The development of pink colouration due to TTC in a well indicated bacterial growth, and the absence of the colour was taken to mean inhibition of bacterial growth. The first well of the micro plate was the control and did not have any plant extract. The MIC value was noted at the well, where no colour was manifested. Furthermore, 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 nutrient agar plate was observed, was noted as the MBC value.[11]

Qualitative phytochemical analyses

Biochemical tests were performed with methanol extracts of used medicinal plants for alkaloids, carbohydrates, saponins, flavonoids, steroids/terpenes, tannins and glycosides, as detailed.[20]

Thin layer chromatography

The methanol extract of the *Solanum xanthocarpum* was subjected to thin layer chromatography (TLC) analysis, to separate the polar and non-polar phytocompounds,[21] for confirmation. Two solvent systems were used during TLC work, (1) ethyl acetate: acetic acid: formic acid, 8:1.5:0.5, v/v, and (2) chloroform: methanol, 8.5:1.5, v/v for separation. The leaf-extract of *S. xanthocarpum* was further used for TLC analysis. Precoated aluminium-backed silica gel GF254 plates (MERCK, Germany) were used.

Molecular docking

The structure of the target protein, β-lactamase enzyme (PDB ID: 1BT5) of *E. coli* resistant to β-lactam antibiotics, was retrieved from Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do), and in addition, the structure and relevant information of 7 phytochemicals of *S. xanthocarpum* were retrieved from the PubChem (http://www.ncbi.nlm.nih.gov/pccompound) database, for a docking study. The AutoDock 4.2.6 and ArgusLab 4.0.1 software were used for the molecular docking process; and the Pylon and Discovery studio software were used for the protein-ligand interactions. In this study, the β-lactamase enzyme is the largest molecule against which the phytochemicals and antibiotics were docked, individually as ligands. The obtained docking score values could be used to predict the individual efficacy of each ligand against the enzyme. Eventually, the pathogen *E. coli* could be computationally predicted to be controlled by the phytochemicals, in parallel with the efficacy of the antibiotic gentamicin.

Results

Ethnomedicinal information on 21 reported medicinal plants were documented along with the details of the modalities of crude extracts as medicine for several ailments, which were used by local ethnic aborigine tribes (Table 1). All of these plants are in use for treating cough, diarrhoea, diabetes, bronchitis, fever, gum bleed, ulcers, killing worms, constipation, piles and urinary tract infections. However, parts of the majority of the plants (17 of 21 plants) are not edible as medicines.

Identification of pathogenic bacterial strains was performed with gross colony morphologies (Table 2) and biochemical test results (Table 3), along with those of the corresponding MTCC strains in reference. For example, the GP bacterium, *S. aureus*, was catalase and coagulase positive, while the GP bacterium *E. faecalis* was catalase and coagulase negative along with positivity to the bile

Table 1: Ethnomedicinal uses of 21 plants from aborigines of Kalahandi district, Odisha.

Sl. No	Plant name; specimen number	Family	Local name; parts used	Ethnomedicinal uses	Yield of ME (mg)
1.	*Abelmoschus Crinitus* Wall.; KLD126	Malvaceae	*Bana bhendi*; Root, leaf.	Root is used in dysentery and diarrhoea. Leaves are used for treating diabetes, jaundice, cholera, asthma.	0.25
2.	*Ageratum conyzoides* L.; KLD139	Asteraceae	*Pokasungha*; leaf, root	Leaf paste is used for allergy, scabies and anti-infection. The juice of the root is used for dysentery.	0.19
3.	*Annona squamosa* L. (Ed); KLD105	Annonaceae	*Sitaphala*; leaf, bark.	Bark is used for urinary infections. Leaf is used for diarrhoea, fever, inflammation, blood vomiting, cough, vomiting, wounds and ulcers.	0.20
4.	*Aerva lanata* (L.) Juss. Ex Sch. (Ed); KLD187	Amaranthaceae	*Paunsia*; leaf, root	Leaf and root is used in cough, painful discharge of urine, headache, liver congestion, jaundice.	0.23
5.	*Buchanania latifolia* Spreng.; KLD239	Anacardiaceae	*Chara*, leaf	Leaf is used as vermifuge, antiseptic and chicken pox and acne.	0.19
6.	*Cardiospermum halicacabum* L. KLD274	Sapindaceae	*Mandibatha*; leaf, whole plant	Leaf is used as arthritis, pain relief, ear ache, purulent discharge, dandruff and diarrhoea.	0.23
7.	*Careya arborea* Roxb.; KLD278	Lecythidaceae	*Kumbhi*, leaf, bark	Leaf is used to protect stomach from ulcer formation. Bark is useful in diarrhoea, blood dysentery.	0.26
8.	*Cleome viscosa* L.; KLD282	Capparaceae	*Banasorisha*; leaf	Leaf is used for eyesore, piles, dysentery and rheumatism.	0.23
9.	*Curcuma aromatic* Salisb.; KLD296	Zingiberaceae	*Bana haladi*; Leaf, rhizomes	Leaf is used for skin infection and anthelmintic. Rhizomes are used for burning of stomach.	0.21
10.	*Cuscuta reflexa* Roxb.; KLD299	Cuscutaceae	*Nirmuli*; whole plant	It is used in the treatment of diarrhoea, dysentery, rheumatism swellings, and joint pain.	0.24
11.	*Dioscorea bulbifera* L.; KLD304	Dioscoreaceae	*Pitta alu*; leaf, rhizome	Leaf is used in skin and scabbing activities. Rhizomes are used in piles, cough and constipation.	0.27
12.	*Diospyros peregrine* (Gaertn.) Guerke; KLD319	Ebenaceae	*Mankada kendu*; Fruit, leaf	Fruit juice is used for diarrhoea, dysentery. Leaves are used in infection, asthma and ulcers.	0.28
13.	*Glycosmis arborea* (Roxb.) DC; KLD502	Rutaceae	*Bana lembu*; leaf	Leaves are useful for dysentery, piles, and infections.	0.25
14.	*Holarrhena pubescens* (Buch. Ham.) Wall.exDon.; KLD359	Apocynaceae	*Keruon*; leaf, bark	Bark and leaves are used in treatment of piles, skin diseases, urinary troubles, diarrhoea.	0.19
15.	*Nicotiana tabacum*L.; KLD664	Solanaceae	*Dhuanpatra*; leaf	In a snake bite, the leaf is taken in such a way that poison does not spread.	0.27
16.	*Ocimum tenuiflorum* L. (Ed); KLD541	Lamiaceae	*Karpur tulsi*; leaf, whole plant	Leaves are useful for cold, cough, tonsillitis, fever, asthma and bronchitis.	0.29
17.	*Phoenix pusilla* Roxb. (Ed); KLD280	Arecaceae	*Banakhajuri*; leaf, flower, root	Leaves are useful in fever. Flowers are useful in asthma, inflammations. Its root is used for asthma, bronchitis.	0.17
18.	*Senna alata*(L.) Roxb.; KLD 337	Fabaceae	*Dadmari*; leaf, flower	Leaves are used for ring worm, skin infection and constipation. Flower used for eczema.	0.14
19.	*Solanum xanthocarpum* Schrad & Wend. KLD772	Solanaceae	*Bhejibaigana*; leaf, flower	Leaf juice with black pepper and honey is taken on an empty stomach for a week to cure cough and tonsillitis. Flowers fried in fat are chewed everyday to cure asthma.	0.29
20.	*Toddalia asiatica* (L.) Lam.; KLD746	Rutaceae	*Tundipoda*; leaf	Leaf is useful in skin diseases, burning sensations, dry cough, dysentery, inflammation, boils, and ringworm infection.	0.18
21.	*Ziziphus oenoplia* (L.) Mill. (Ed); KLD694	Rhamnaceae	*Kanteikoli*; leaf, bark	Leaves used for wound healing, skin infection. Bark used for dysentery and sore throats.	0.20

Ed, edible; ME, methanol extract.

esculin test. Among the GN strains, *E. coli* (Figure 1) cells were positive to tests for catalase, indole, MR and nitrate production, while in the TSI test, the production of acid in the slant and gas in the butt of the tube occurred, and the *E. coli* cells were negative to other biochemical tests. *P. aeruginosa* was identified by its positivity to catalase, oxidase, citrate, urease and nitrate tests, while it had negativity to the indole, MR and VP tests. The remaining bacteria were also identified using similar features (Tables 2 and 3).

Table 2: Colony characteristics of two Gram-positive and eight Gram-negative pathogenic bacteria isolated from clinical samples.

Bacterium	MTCC strain	Media used	Colony characteristics
Enterococcus faecalis[a]	439	Blood agar	Grey coloured, round, gamma haemolytic colonies
Staphylococcus aureus[a]	7443	Blood agar	Medium to large, smooth, entire, slightly raised, creamy yellow, with green/beta haemolytic colonies
		Nutrient agar	As above without haemolytic activity
Acinetobacter baumannii	1425	Nutrient agar	Colourless smooth, opaque, raised and pinpoint
		MacConkey agar	Colourless smooth, opaque, raised, NLF
		CLED agar	Blue coloured opaque raised NLF
Citrobacter freundii	1658	MacConkey agar	Late LF light pink after 48 h
Enterobacter aerogenes	2990	Blood agar	White convex with gamma—haemolysis
		MacConkey agar	LF, mucoid
Escherichia coli	443	Nutrient agar	Flat dry, irregular
		MacConkey agar	LF, flat dry pink, irregular
		EMB agar	Purple coloured, flat dry, irregular colonies, with metallic green colour.
		Blood agar	Swarms on blood agar with beta—haemolysis
		CLED agar	Translucent blue
Klebsiella pneumoniae	4031	MacConkey agar	LF, pink, mucoid
		CLED agar	Yellow mucoid
Proteus mirabilis	739	MacConkey agar	NLF light pink after 48 h
		Blood agar	Swarms on blood agar with beta—haemolysis
		CLED agar	Translucent blue
Proteus vulgaris	1771	Blood agar	Swarms on blood agar with beta—haemolysis
		CLED agar	Translucent blue
Pseudomonas aeruginosa	1688	Nutrient agar	Large, irregular opaque with bluish green pigment

[a] Gram-positive bacteria; MTCC, microbial type culture collection; CLED, cysteine lactose electrolyte deficient; EMB, eosin methylene blue; LF, lactose fermenting; NLF, non-lactose fermentation.

Table 3: Biochemical identifications of the isolated Gram-positive and Gram-negative bacteria.

Bacterium	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	Bile esculin
E. faecalis	−	Nd	−	Nd	Nd	Nd	Nd	Nd	Nd	Nd	+
S. aureus	+	Nd	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
A. baumannii	+	−	Nd	−	−	+	+	V	Nd	−	Nd
C. freundii	+	−	Nd	−	+	−	+	−	A/A H_2S	+	Nd
E. aerogenes	+	−	Nd	−	−	+	+	−	A/A	+	Nd
E. coli	+	−	Nd	+	+	−	−	−	A/AG	+	Nd
K. pneumoniae	+	−	Nd	−	−	+	+	+	A/AG	+	Nd
P. mirabilis	+	−	Nd	−	+	−	+	+	K/AH_2S	+	Nd
P. vulgaris	+	−	Nd	+	+	−	+	+	K/AH_2S	+	Nd
P. aeruginosa	+	+	Nd	−	−	−	+	+	Nd	+	Nd

MR, methyl red test; VP, Voges—Proskauer test; TSI, triple sugar iron test; V, variable; A/A, acid in slant and butt; A/AG H_2S, acid in slant and butt with H_2S gas production; A/AG, acid in slant and butt with gas production; K/A H_2S, alkali in slant and butt with H_2S gas production; Nd, not done; +, positive; −, negative.

The antibiotic profile of each pathogenic bacterium was determined using specified antibiotic discs (Table 4). *E. faecalis* was resistant to 16 and *S. aureus* was resistant to 12 of 18 prescribed antibiotics. Among the 8 GN isolates, *A. baumannii* was resistant to 9 antibiotics, *C. freundii* and *K. pneumoniae* were resistant to 10 antibiotics, *E. coli* and *P. vulgaris* were resistant to 11 antibiotics, and *E. aerogenes* was resistant to 13 of the total 14 antibiotics used. Details of individual antibiograms of individual bacterium were determined (Table 4). Clearly, all of the isolated bacterial strains were floridly MDR.

Diameter values of zones of inhibition due to methanol extracts from the leaves of 21 plants against MDR strains of 10 bacteria were recorded (Table 5, Figure 2). The most effective 5 plants, which caused the size of the zone of inhibition (an effective controlling capacity) to be 21—27 mm, were *Buchanania latifolia, Careya arborea, Ocimum tenuiflorum, Senna alata* and *S. xanthocarpum,* for any GP or GN MDR bacterial strain. The methanol leaf-extract of the plant, *B. latifolia*, had the highest size for the zone of inhibition of 25 mm, against *S. aureus*, and the lowest value of 15 mm against *P. vulgaris*. The methanol leaf-extract of *C. arborea* had the highest size of the zone of inhibition, 25 mm, against *S. aureus,* which had the lowest value of 14 mm against *C. freundii*. The methanol leaf-extract of *O. tenuiflorum* had the highest size of the zone of inhibition, 26 mm, against *S. aureus,* and the lowest value of 14 mm was

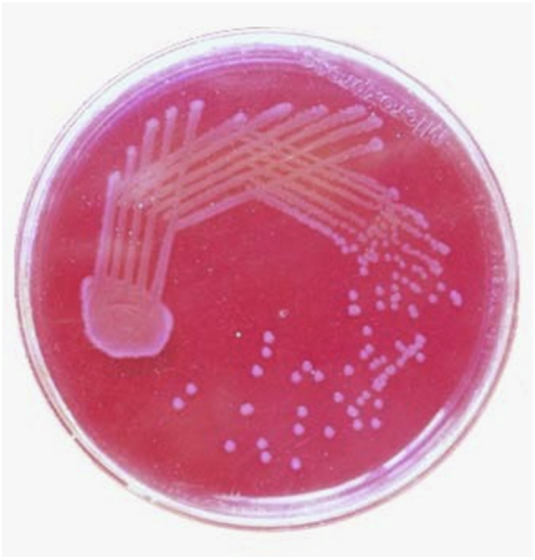


Figure 1: *Escherichia coli* in MacConkey agar.

against *P. mirabilis*. The methanol-extract of *S. alata* had the highest value of the size of the zone of inhibition, which was 25 mm, against *E. coli* and the smallest size of the zone of inhibition was 13 mm against *A. baumannii*. The methanol leaf-extract of *S. xanthocarpum* had the highest size of the zone of inhibition, which was 25 mm, against *E. coli*, and the lowest value of 18 mm was against *E. aerogenes*. The methanol leaf-extracts of the plants *Abelmoschus crinitus*, *Ageratum conyzoydes*, *Annona squamosa*, *Curcuma aromatica*, *Dioscorea bulbifera*, *Diospyros peregrine*, *Glycosmis arborea, Holarrhena pubescens*, *Cuscuta reflexa* and *Ziziphus oenoplia* showed moderate control capacity. Methanol leaf-extracts of the remaining 6 plants, *Aerva lanata*, *C. halicacabum*, *C. viscose*, *C. reflexa*, *Phoenix pusilla* and *Toddalia asiatica,* were comparatively less effective in controlling the MDR strains of the isolated pathogenic bacteria.

Among 21 plants, *S. xanthocarpum* had 0.67 mg/ml as the lowest MIC value and 1.51 mg/ml as the lowest MBC value against *E. coli*, and this plant had the MIC value of 3.41 mg/ml and the MBC value of 4.27 mg/ml for *E. aerogenes*. A lower MIC/MBC value signifies that a minimum amount of

Table 4: Prescribed antibiotic susceptibility results against isolated Gram-positive and Gram-negative bacteria.

Bacterium	Susceptibility to prescribed antibiotics																	
	Amino-glycosides		β-lactams				Cephalo-sporins		Fluoroquinolones				Glyco-peptides		Lincos-amide	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	MS	R	R	R	R	R	R	R	R	R	S	R	R	R	R
S. aureus	R	R	R	MS	MS	R	R	R	R	R	R	R	MS	MS	S	R	R	S
A. baumannii	R	R	R	S	ND	R	R	R	R	R	S	S	ND	ND	ND	S	R	S
C. freundii	S	R	R	R	ND	R	R	R	S	R	R	MS	ND	ND	ND	R	R	S
E. aerogenes	R	R	R	R	ND	R	R	R	R	R	R	R	ND	ND	ND	R	MS	R
E. coli	R	R	R	R	ND	S	R	R	R	R	R	R	ND	ND	ND	S	R	S
K. pneumoniae	R	R	R	R	ND	R	R	R	R	R	S	S	ND	ND	ND	R	MS	S
P. mirabilis	S	R	R	R	ND	S	R	R	S	R	S	MS	ND	ND	ND	R	S	R
P. vulgaris	R	R	R	S	ND	R	R	S	S	R	R	S	ND	ND	ND	S	R	R
P. aeruginosa	R	R	R	R	ND	R	R	R	S	R	R	MS	ND	ND	ND	R	R	S

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; Le, levofloxacin 5; Lz, linezolid 30; Of, ofloxacin 5; Ox, oxacillin 1; Pt, piperacillin/tazobactam 100/10; Nx, norfloxacin 10; Tei, teicoplanin 5; Va, vancomycin 30.

Table 5: Antibacterial activity of methanol leaf extracts of 21 plants by the agar-well diffusion method, measured as the size of the zone of inhibition (mm).

Bacteria	Diameter size of zone of inhibition by methanol leaf extracts of plants																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Ge
E. faecalis	17	19	19	14	24	18	22	17	18	16	19	18	18	19	17	21	19	20	23	18	18	30
S. aureus	18	18	16	14	25	14	25	18	17	14	20	14	14	17	18	26	15	24	21	17	17	28
A. baumannii	17	21	20	18	20	–	17	18	18	13	17	20	16	–	17	17	13	13	19	18	19	22
C. freundii	20	–	17	–	16	–	14	14	–	–	16	14	14	18	19	18	–	15	19	20	20	26
E. aerogenes	16	17	19	–	17	13	18	16	19	18	17	15	19	–	13	20	18	18	18	18	–	24
E. coli	21	14	14	19	19	15	20	15	16	17	18	18	16	16	14	16	–	25	25	–	18	26
K. pneumoniae	17	17	–	17	20	13	15	13	18	18	20	16	19	18	18	19	18	21	23	19	19	26
P. mirabilis	18	18	14	17	17	–	19	–	17	19	18	–	18	17	17	14	–	17	19	–	18	28
P. vulgaris	14	–	20	–	15	16	16	18	19	18	16	17	–	–	14	17	16	15	19	18	16	26
P. aeruginosa	19	17	17	19	21	18	23	18	20	20	18	19	17	20	14	24	17	21	20	–	21	28

Ge; gentamicin 30 mg/ml used as the control. The numbers 1 to 21 are serial numbers of plants given in Table 1; the values are the measurements of the zone of inhibition in triplicate due to methanol leaf extracts. The "–" sign denotes no activity.

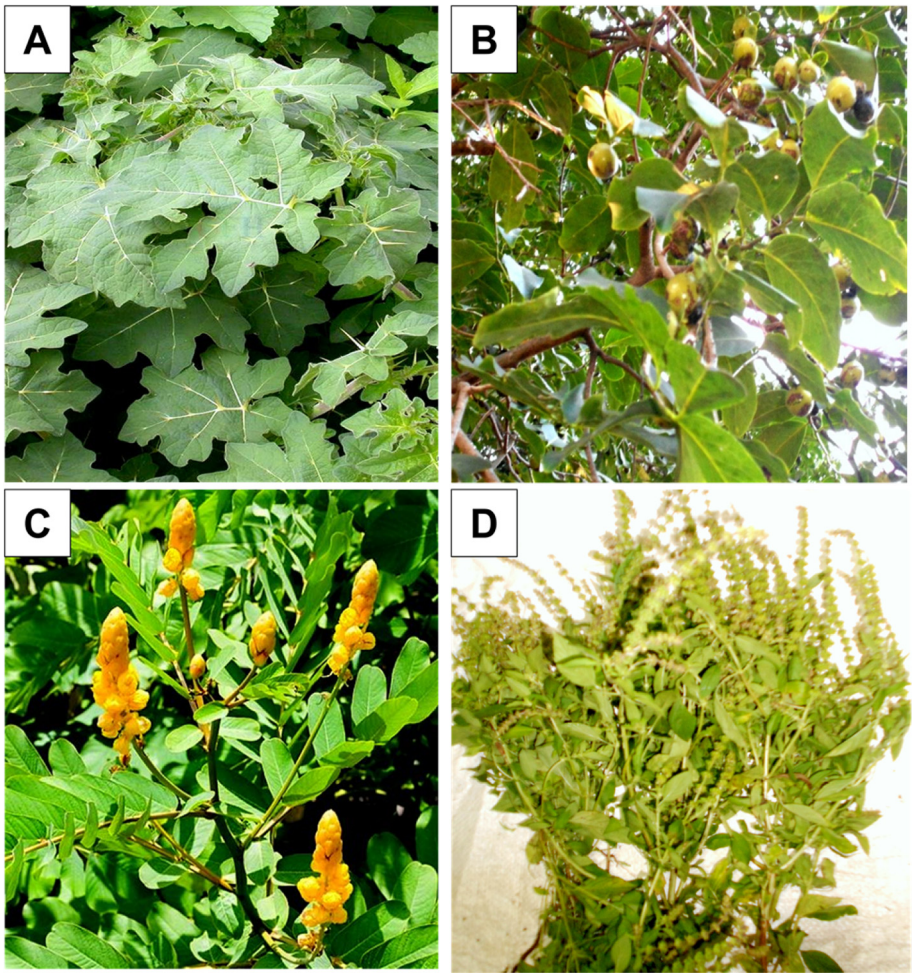


Figure 2: A, *Solanum xanthocarpum*; B, *Diospyros peregrine*; C, *Senna alata*, D, *Ocimum tenuiflorum*.

plant extract was used, whereas a higher value signifies the use of a larger amount of plant extract for the control of any bacterium. The MIC and MBC values due to the methanol extracts of the remaining plants were recorded (Table 6a, b, c).

Qualitative phytochemical analysis was performed for these 21 plants. All of these plants contained phytochemicals, alkaloids, glycosides flavonoids, carbohydrates, terpenoids, steroids, and tannins, which could be attributed to their recorded significant antibacterial activities (Table 7). Using the first solvent system, 5 spots from the methanol extracts of *S. xanthocarpum* were identified in the TLC plate, which had the following retardation factor (R_f) values: 0.19, 0.22, 0.32, 0.63 and 0.74. With the second solvent system, there

Table 6a: MIC and MBC values by methanol leaf extracts of the first seven plants against pathogenic bacteria (mg/ml).

Bacterium	MIC and MBC values of plant numbers 1 to 7													
	1		2		3		4		5		6		7	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	4.27	9.63	3.41	4.27	3.41	4.27	9.63	21.67	0.67	1.51	3.41	4.27	1.51	3.41
S. aureus	3.41	4.27	3.41	4.27	4.27	9.63	9.63	21.67	0.67	1.51	9.63	21.67	0.67	1.51
A. baumannii	4.27	9.63	1.51	3.41	3.41	4.27	4.27	9.63	3.41	4.27	–	–	4.27	9.63
C. freundii	3.41	4.27	–	–	4.27	9.63	–	–	4.27	9.63	–	–	9.63	21.67
E. aerogenes	4.27	9.63	4.27	9.63	3.41	4.27	–	–	4.27	9.63	9.63	21.67	3.41	4.27
E. coli	1.51	3.41	4.27	9.63	4.27	9.63	3.41	4.27	3.41	4.27	4.27	9.63	3.41	4.27
K. pneumoniae	4.27	9.63	4.27	9.63	–	–	4.27	9.63	3.41	4.27	9.63	21.67	4.27	9.63
P. mirabilis	3.41	4.27	3.41	4.27	9.63	21.67	4.27	9.63	4.27	9.63	–	–	3.41	4.27
P. vulgaris	9.63	21.67	–	–	3.41	4.27	–	–	4.27	9.63	4.27	9.63	4.27	9.63
P. aeruginosa	3.41	4.27	4.27	9.63	4.27	9.63	3.41	4.27	1.51	3.41	3.41	4.27	1.51	3.41

Table 6b: MIC and MBC values by methanol leaf extracts of the second seven plants against pathogenic bacteria (mg/ml).

Bacterium	MIC and MBC values of plant numbers 8 to 14													
	8		9		10		11		12		13		14	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	4.27	9.63	3.41	4.27	4.27	9.63	3.41	4.27	3.41	4.27	3.41	4.27	3.41	4.27
S. aureus	3.41	4.27	4.27	9.63	9.63	21.67	3.41	4.27	9.63	21.67	9.63	21.67	4.27	9.63
A. baumannii	3.41	4.27	3.41	4.27	9.63	21.67	4.27	9.63	3.41	4.27	4.27	3.41	–	–
C. freundii	9.63	21.67	–	–	–	–	4.27	9.63	9.63	21.67	9.63	21.67	3.41	4.27
E. aerogenes	4.27	9.63	3.41	4.27	3.41	4.27	4.27	9.63	4.27	9.63	3.41	4.27	–	–
E. coli	4.27	9.63	4.27	9.63	4.27	9.63	3.41	4.27	3.41	4.27	4.27	3.41	4.27	9.63
K. pneumoniae	9.63	21.67	3.41	4.27	3.41	4.27	3.41	4.27	4.27	9.63	3.41	4.27	3.41	4.27
P. mirabilis	–	–	4.27	9.63	3.41	4.27	3.41	4.27	–	–	3.41	4.27	4.27	9.63
P. vulgaris	3.41	4.27	3.41	4.27	3.41	4.27	4.27	9.63	4.27	9.63	–	–	–	–
P. aeruginosa	3.41	4.27	3.41	4.27	3.41	4.27	3.41	4.27	3.41	4.27	4.27	3.41	3.41	4.27

Table 6c: MIC and MBC values by methanol leaf extracts of the third seven plants against pathogenic bacteria (mg/ml).

Bacterium	MIC and MBC values of plant numbers 15 to 21													
	15		16		17		18		19		20		21	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
E. faecalis	4.27	9.63	1.51	3.41	3.41	4.27	3.41	4.27	1.51	3.41	3.41	4.27	3.41	4.27
S. aureus	3.41	4.27	0.67	1.51	4.27	9.63	0.67	1.51	1.51	3.41	4.27	9.63	4.27	9.63
A. baumannii	4.27	9.63	4.27	9.63	9.63	21.67	9.63	21.67	3.41	4.27	3.41	4.27	3.41	4.27
C. freundii	3.41	4.27	3.41	4.27	–	–	4.27	9.63	3.41	4.27	3.41	4.27	3.41	4.27
E. aerogenes	9.63	21.67	3.41	4.27	3.41	4.27	3.41	4.27	3.41	4.27	3.41	4.27	–	–
E. coli	9.63	21.67	4.27	9.63	–	–	0.67	1.51	0.67	1.51	–	–	3.41	4.27
K. pneumoniae	3.41	4.27	3.41	4.27	3.41	4.27	1.51	3.41	1.51	3.41	3.41	4.27	3.41	4.27
P. mirabilis	4.27	9.63	9.63	21.67	–	–	4.27	9.63	3.41	4.27	–	–	3.41	4.27
P. vulgaris	9.63	21.67	4.27	9.63	4.27	9.63	4.27	9.63	3.41	4.27	3.41	4.27	4.27	9.63
P. aeruginosa	9.63	21.67	0.67	1.51	4.27	9.63	1.51	3.41	3.41	4.27	–	–	1.51	3.41

were 4 rising spots, which had the R_f values of 0.27, 0.38, 0.54 and 0.72, in the TLC plate.

The molecular docking of the β-lactamase−phytochemical interaction was aimed at locating the potentiality of some of the leading phytochemicals. Phytocompounds of *S. xanthocarpum,* solasodine and stigmasterol glucoside had the highest docking score values, −10.868 kcal/mol and −10.439 kcal/mol, respectively, against β-lactamase of *E. coli*, as the target protein (Figure 3). It was recorded that the seven phytochemicals could be arranged as being effective in their decreasing order of docking score value, solasodine (−10.868) > stigmasterol glucoside (−10.439) > esculin (−8.744) > apigenin (−8.724) > lupeol (−8.495) > scopoletin (−8.399) > caffeic acid (−8.341), which is based on the negativity of the docking score values; a more negative docking score value is selected to be the more active compound (Table 8). Thus, the development of plant derivative/phytochemical(s) as antibacterial agents could be indicated by using the tools of bioinformatics, thus eliminating the time and resources of the "hit-and-miss" method required for drug development. Thus, this computational attempt to use the tools of bioinformatics in identifying suitable phytochemical(s), especially from *S. xanthocarpum,* for use as alternative agents from plant derivative drugs to address suitably and overcome antibiotic resistance (see Table 8).

Discussion

In Indian *Ayurveda*, Chinese traditional medicine (TM), and similarly in several countries, a large number of plants, based on ethnobotanical information, are in use as crude medicines locally, while a plethora of pure phytochemicals have lent themselves to the modern drug development process against infectious and non-infectious diseases.[22] Today, the concept of TM as complementary and alternative medicine (CAM) has been popular worldwide.[23,24] Consequently, the World Health Organization (WHO) has issued mandates that describe plants as the best source of a variety of drugs.[24] Indeed, several formulations of crude drugs of individual plants are available on the market worldwide.

In the present study, plants such as *A. squamosa* and *O. tenuiflorum* have antimicrobial and antioxidant activities; such plants are suitable candidates for being used as CAM. The complexity of phytochemicals in a crude extract is due to flavonoids, saponins, glycosides, terpenoids, steroids and phenols and other compounds; flavonoids are less toxic than alkaloids, generally.[25] Thus, an edible plant, such as *A. squamosa*, *A. lanata*, *O. tenuiflorum*, *P. pusilla*, or *Z. oenoplia,* should have limited alkaloid content, and basically, an edible plant contains a substantial amount of flavonoids holistically. In acute toxicity or host toxicity

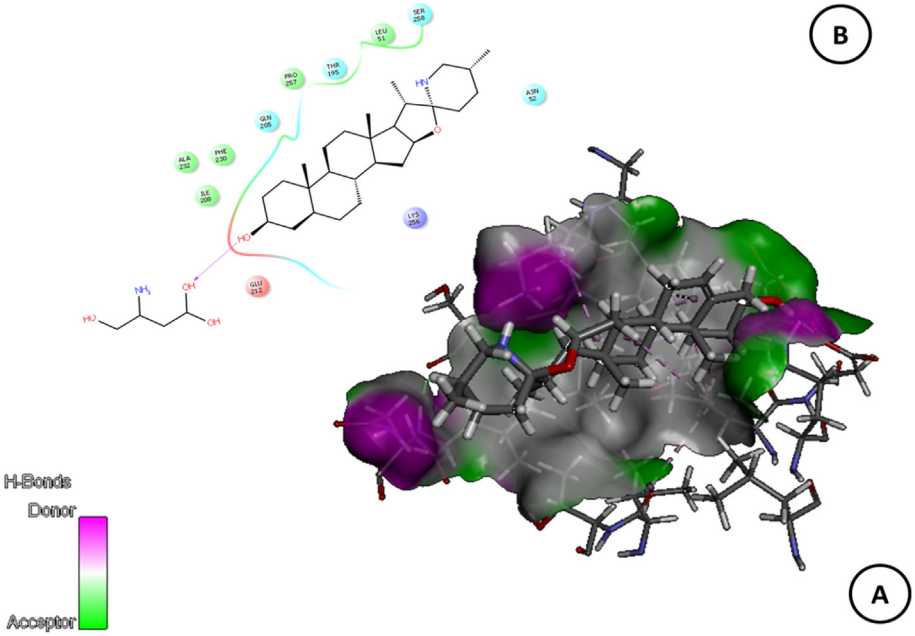


Figure 3: A, Three-dimensional structure of protein-ligand interaction of solasodine isolated from *S. xanthocarpum* against β-lactamase enzyme of *E. coli* (PDB ID: 1BT5) with surface view using Discovery studio Visualizer 3.1 software; B, the same without surface view with solasodine interacting with target enzyme, β-lactamase.

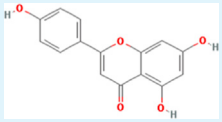
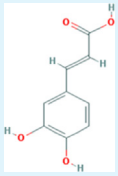
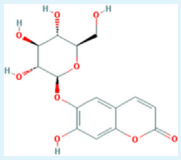
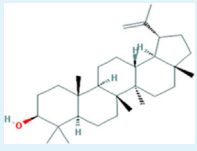
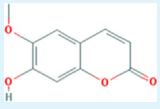
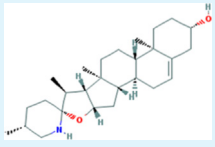
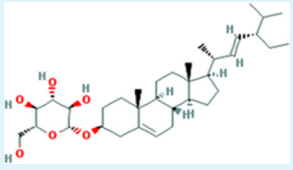
Table 7: Qualitative phytochemical analysis of the methanol leaf extracts of the 21 medicinal plants.

Plants	Alkaloids	Glycosides	Terpenoids	Carbohydrates	Tannins	Flavonoids	Steroids
A. crinitus	+	−	+	+	+	+	+
A. conyzoides	+	−	+	−	+	+	+
A. squamosa	+	+	+	−	+	+	−
A. lanata	+	−	+	+	+	+	+
B. latifolia	−	+	+	+	+	+	+
C. halicacabum	+	+	+	+	−	+	+
C. arborea	+	+	−	−	−	−	+
C. viscosa	+	+	+	−	+	−	−
C. aromatica	−	−	+	+	−	−	−
C. reflexa	+	−	−	+	−	+	−
D. bulbifera	+	+	−	−	+	+	−
D. peregrina	+	−	−	−	+	+	−
G. arborea	+	−	+	−	−	+	+
H. pubescens	−	+	−	+	−	+	−
N. tabacum	+	+	−	−	+	+	−
O. tenuiflorum	+	−	+	−	+	+	−
P. pusilla	+	−	+	+	+	+	+
S. alata	−	+	+	−	−	−	+
S. xanthocarpum	+	+	+	+	+	+	+
T. asiatica	−	−	+	−	+	+	+
Z. oenoplia	+	−	+	+	−	+	+

"+" sign denotes presence, and "−"sign denotes absence of the compound in a plant.

studies in mammals, acting in accordance with pharmacological guidelines is the most suitable approach for an individual crude extract of a plant. Obviously, phyto-drugs are cost effective in comparison with chemical drugs, and those could be promoted as CAM after host toxicity testing. An example of the *in silico* use of phyto-chemicals enhances the antibacterial activity/potentiality of rout antibiotics acting as a drug efflux pump.[26] In a study, aqueous and methanol extracts of the plant, *Grewia serrulata*, had been reported repeatedly in Wistar Albino rats in toxicity testing attempts, and this plant was taken to be non-toxic.[27] Inherently, alkaloids are more toxic, and flavonoids are curatives, in general. Based on specific requirements, any of the phytochemicals are useful as drugs; for example, plant alkaloids are used against malaria, while flavonoids are used against hyperglycaemia.

Table 8: Structure of and information on the selected isolated bioactive compounds of *S. xanthocarpum* along with individual protein-ligand docking score values against β-lactamase of *E. coli*.

Name; (chemical class)	Information	Chemical structure	Docking score (kcal/mol)
Apigenin; (F)	MW: 270.2369 g/mol MF: $C_{15}H_{10}O_5$ H-bond donor: 3 H-bond acceptor: 5 PubChem ID: CID 5280443		−8.724
Caffeic acid; (P)	MW: 180.15742 g/mol MF: $C_9H_8O_4$ H-bond donor: 3 H-bond acceptor: 4 PubChem ID: CID 689043		−8.341
Esculin; (C)	MW: 340.28214 g/mol MF: $C_{15}H_{16}O_9$ H-Bond donor: 5 H-Bond acceptor: 9 PubChem ID: CID 5281417		−8.744
Lupeol; (T)	MW: 426.7174 g/mol MF: $C_{30}H_{50}O$ H-Bond donor: 1 H-Bond acceptor: 1 PubChem ID: CID 259846		−8.495
Scopoletin; (C)	MW: 192.16812 g/mol MF: $C_{10}H_8O_4$ H-bond donor: 1 H-bond acceptor: 4 PubChem ID: CID 5280460		−7.399
Solasodine; (A)	MW: 413.63582 g/mol MF: $C_{27}H_{43}NO_2$ H-Bond donor: 2 H-Bond acceptor: 3 PubChem ID: CID 442985		−10.868
Stigmasterol glucoside; (S)	MW: 574.83142 g/mol MF: $C_{35}H_{58}O_6$ H- bond donor: 4 H- bond acceptor: 6 PubChem ID: CID 6602508		−10.439

A, alkaloid; C, coumarin, F, flavonoid; P, phenol; S, steroid; T, terpenoid and H-bond donor, hydrogen bond acceptor; H-bond acceptor, hydrogen bond donor; MF, molecular formula; MW, molecular weight; chemical structures were retrieved from PubChem database.

In the present docking attempt, one alkaloid and a sterol were the most effective ligands.

An example of the synergistic use of methanol leaf-extract of *Combretum albidum* and antibiotic ceftriaxone in controlling ceftriaxone-resistant of *P. aeruginosa in vitro* was demonstrated.[28] Similarly, extracts of 7 Cameroonian spice plants were described as having synergistic effects against the antibiotic-resistant pathogenic bacteria, *Providencia stuartii*, *P. aeruginosa, K. pneumoniae*, *E. coli*, *E. aerogenes* and *Enterobacter cloacae*.[29] Thus, the most effective plants could be used synergistically with mainstream medicine against MDR bacteria.

Currently, the drug discovery process has been revolutionized by the computational molecular docking method.[30] For this purpose, the target molecule against which a suitable drug is to be aimed is retrieved from the protein database as the 'three-dimensional (3D) structure'. If necessary, the structure of the target protein could be modified computationally. However, the target protein is basically a larger molecule against which possible control agents such

as ligands are docked. The target-ligand docking interaction involves docking score values that are expressed as kcal/mol.[31] In the present system's docking study, the β-lactamase enzyme was the target protein against which the phytochemicals apigenin, caffeic acid, esculin, lupeol, scopoletin, solasodine and stigmasterol glucoside were docked. The β-lactam group of antibiotics is frequently used in the clinic for the control of infectious episodes from GN bacteria. Frequent reports of resistance to these antibiotics are due to the capability of the production of β-lactamases by resistant bacterial strains, which degrade the β-lactam ring. Thus, this enzyme is a putative target for drug development modules.[32] Phytochemicals, for example, alkaloid, phenol and terpenoids, inhibit any specific bacterial target and work against the cell wall; and flavonoids and tannins target the inhibition of a specific bacterial enzyme.[33] Moreover, *S. xanthocarpum* chemicals rising as spots on the TLC plate might be effectively capable of controlling the MDR bacteria. From the docking score values, the ligand with the minimum docking score values was solasodine and stigmasterol glucoside of *S. xanthocarpum,* which were taken as suitable ligands, in other words, as the leading antibacterials.

Conclusions

Hidden ethnomedicinal information of aborigines could be further validated, and the most effective plant(s) could be exploited for the preparation of complementary drugs. From 21 plants that were monitored for antimicrobial activity, 5 plants may be useful as sources of non-microbial antibacterials; specifically, *S. xanthocarpum* was observed as the best plant based on the *in vitro* study. Blithely, from the molecular docking approach, its solasodine and stigmasterol glucoside were the most promising phytochemicals against β-lactamase.

Contributions

SS Swain conducted the experiments with the supervision of RN Padhy. SS Swain prepared the draft manuscript, and RN Padhy edited it.

Ethical approval

Not required.

Conflict of interest statement

The authors have no conflict of interest to declare.

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References

1. Hawkey PM. Multidrug-resistant gram-negative bacteria: a product of globalization. **J Hosp Infect 2015**; 89(4): 241–247.
2. Delgado-Valverde M, Sojo-Dorado J, Pascual A, Rodríguez-Baño J. Clinical management of infections caused by multidrug-resistant *Enterobacteriaceae*. **Ther Adv Infect Dis 2013**; 1(2): 49–69.
3. Zervosen A, Sauvage E, Frère J-M, Charlier P, Luxen A. Development of new drugs for an old target — the penicillin binding proteins. **Molecules 2012**; 17(11): 12478–12505.
4. Laxminarayan R, Duse A, Wattal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance-the need for global solutions. **Lancet Infect Dis 2013**; 13(12): 1057–1098.
5. Gu WJ, Wang F, Tang L, Bakker J, Liu JC. Colistin for the treatment of ventilator-associated pneumonia caused by multidrug-resistant gram-negative bacteria: a systematic review and meta-analysis. **Int J Antimicrob Agent 2014**; 44(6): 477–485.
6. Rojo D, Gosalbes MJ, Ferrari R, Pérez-Cobas AE, Hernández E, Oltra R, et al. Clostridium difficile heterogeneously impacts intestinal community architecture but drives stable metabolome responses. **Int Soc Microb Ecol 2015**; 9: 2206–2220.
7. Baltrus DA. Exploring the costs of horizontal gene transfer. **Trends Ecol Evol 2013**; 28(8): 489–495.
8. Aminov RI. Horizontal gene exchange in environmental microbiota. **Front Microbiol 2011**; 2: 1–19.
9. Muscedere JG, Day A, Heyland DK. Mortality, attributable mortality, and clinical events as end points for clinical trials of ventilator-associated pneumonia and hospital-acquired pneumonia. **Clin Infect Dis 2010**; 51(1): S120–S125.
10. Dubey D, Rath S, Sahu MC, Pattnaik L, Debata NK, Padhy RN. Surveillance of infection status of drug resistant *Staphylococcus aureus* in an Indian teaching hospital. **Asian Pac J Trop Dis 2013**; 3(2): 133–142.
11. Sahu MC, Dubey D, Rath S, Panda T, Padhy RN. Monograph: *In vitro* efficacy of 30 ethnomedicinal plants used by Indian aborigines against 6 multidrug resistant gram-positive pathogenic bacteria. **Asian Pac J Trop Dis 2015**; 5(2): 136–150.
12. Rath S, Padhy RN. Prevalence of two multidrug-resistant *Klebsiella* species in an Indian teaching hospital and adjoining community. **J Infect Pub Health 2014**; 7(6): 496–507.
13. Dubey D, Sarangi R, Debata NK, Padhy RN. Detection of metallo-β-lactamase producing *Klebsiella pneumoniae* in a neonatal septicemia. **J Acu Dis 2013**; 2(1): 82–84.
14. Rath S, Padhy RN. Prevalence of fluoroquinolone resistance in *Escherichia coli* in an Indian teaching hospital and adjoining communities. **J Taibah Univ Med Sci 2015**; 10(4): 504–508.
15. Rout S, Dubey D, Panigrahy R, Padhy RN. Surveillance of extended-spectrum β-lactamase producing bacteria in an Indian teaching hospital. **J Taibah Univ Med Sci 2014**; 9(4): 274–281.
16. Mishra MP, Padhy RN. *In vitro* antibacterial efficacy of 21 Indian timber-yielding plants against multidrug-resistant bacteria causing urinary tract infection. **Osong Pub Health Res Perspect 2013**; 4(6): 347–357.
17. Mallik BK, Panda T, Padhy RN. Traditional herbal practices by the ethnic people of Kalahandi district of Odisha, India. **Asian Pac J Trop Biomed 2012**; 2: S988–S994.
18. Mishra MP, Debata NK, Padhy RN. Surveillance of multidrug resistant uropathogenic bacteria in hospitalized patients-an Indian study. **Asian Pac J Trop Biomed 2013**; 3: 315–324.

19. Dubey D, Padhy RN. Antibacterial activity of *Lantana camara* L. against multidrug resistant pathogens from ICU patients of a teaching hospital. **J Herb Med 2013**; 3: 65–75.
20. Sharma V, Paliwal R. Preliminary phytochemical investigation and thin layer chromato-graphy profiling of sequential extracts of *Moringa oleifera* pods. **Int J Green Pharm 2013**; 7(1): 41–45.
21. Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: an integrated approach. **Ayu 2012**; 33(1): 10–19.
22. Yel C. The presentation of complementary and alternative medicine (CAM) in the Turkish press. **Procedia – Soc Behav Sci 2014**; 152: 995–1000.
23. WHO. *Traditional medicine strategy 2014–2023*. Printed in Hong Kong SAR, China, http://apps.who.int/iris/bitstream/10665/92455/1/9789241506090_eng.pdf; 2013 [accessed on 15.01.15].
24. Ozçelik B, Kartal M, Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. **Pharmac Biol 2011**; 49(4): 396–402.
25. Ohene-Agyei T, Mowla R, Rahman T, Venter H. Phytochemicals increase the antibacterial activity of antibiotics by acting on a drug efflux pump. **Microbiologyopen 2014**; 3(6): 885–896.
26. Alviano WS, Alviano DS, Diniz CG, Antoniolli AR, Alviano CS, Farias LM, et al. *In vitro* antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. **Arch Oral Biol 2014**; 53(6): 545–552.
27. Chandiran IS, Jayaveera KN, Karimulla S. Preliminary phytochemical and preclinical toxicitym studies of *Grewia serrulata* DC. **Drug Invent Today 2013**; 5: 267–274.
28. Sahu MC, Patnaik R, Padhy RN. *In vitro* combinational efficacy of ceftriaxone and leaf extract of *Combretum albidum* G. Don against multidrug-resistant *Pseudomonas aeruginosa* and host-toxicity testing with lymphocytes from human cord blood. **J Acu Med 2014**; 4(1): 26–37.
29. Fankam AG, Kuete V, Voukeng IK, Kuiate JR, Pages JM. Antibacterial activities of selected Cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. **BMC Compl Alternet Med 2011**; 11: 104. http://dx.doi.org/10.1186/1472-6882-11-104.
30. Cheng F, Liu C, Jiang J, Lu W, Li W, Liu G, et al. Prediction of drug-target interactions and drug repositioning via network-based inference. **PLoS Comput Biol 2012**; 5: e1002503. http://dx.doi.org/10.1371/journal.pcbi.1002503.
31. Sanders MP, Barbosa AJ, Zarzycka B, Nicolaes GA, Klomp JP, de Vlieg J, Del Rio A. Comparative analysis of pharmacophore screening tools. **J Chem Inf Model 2012**; 52(6): 1607–1620.
32. Bollenbach T. Antimicrobial interactions: mechanisms and implications for drug discovery and resistance evolution. **Curr Opin Microbiol 2015**; 27: 1–9.
33. Simões M, Bennett RN, Rosa EA. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. **Nat Prod Rep 2009**; 26(6): 746–757.