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Impact of Some Ethnomedicinal Plants Extracts Against Screened MDR Strains Isolated from UTI Patients Population of a Tertiary Care Centre in Eastern India

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Abstract

Multidrug resistance bacteria causing urinary tract infections have become a more serious concern nowadays. Gram-positive and gram-negative bacteria are involved in causing urinary- tract infections, and it is more frequent in females as compared to males. Nowadays, there is increasing in antibiotic resistance to almost all available antibiotics, which has become a major therapeutic issue. So, finding ethnomedicinal plants for the treatment of various kinds of infection is a priority. The study aims to assess the antibacterial properties of 3 tropical plants (*Psidium guajava, Syzygium cumini,* and *Punica granatum)* for possible use as antibacterial agents against 4 isolated MDR bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus*). Antibiograms of isolated bacteria were detected by disc- diffusion method, and the antibacterial activity of ethnomedicinal plants was detected by the agar-well diffusion method. Methanol and n-hexane extracts from these 3 plants were used for this study. These 3 ethnomedicinal plants were found to be at least showing 16- 21 mm as a zone of inhibition in lawn culture. Minimum inhibitory concentration and Minimum bactericidal concentration of methanol extracts of 3 plants were recorded. The methanol extract of *Psidium guajava*, *Punica granatum*, and *Szygium cumini* was found to be effective, but *Szygium cumini* was found to be more effective as compared to the *Psidium guajava* and *Punica granatum*.

Keywords: *Escherichia coli, Klebsiella pneumoniae,* Multidrug Resistance Bacteria, *Pseudomonas aeruginosa,* Urinary Tract Infection.

Introduction

In developing countries, fungal infections, pathogenic microorganisms, and bacteria are the leading cause of life-threatening illness, which leads to high illness and death rates, especially in those with weakened immune systems (1). Among human beings, Urinary tract- infection (UTI) is thought to be the most prevalent type of bacterial infection. Based on worldwide surveillance data, following respiratory tract infection UTIs are regarded as 2nd most prevalent type of infection. UTIs account for 25-40% of nosocomial infections (2). It is contemplated as an invasion of microorganisms into the tissue that extends from the renal cortex to the urethral meatus. There are different types of organs that make up the urinary system. This includes types of organs that collect, hold, and release urine from the body (3). It shows different types of symptoms, such as frequent urination, foul smell of urine, pain in the lower abdominal area, and discomfort in the suprapubic area. UTIs are classified into two types, such as complicated and uncomplicated UTIs. Uncomplicated UTIs are commonly involved in lower UTIs and these types of infections can easily be cured by antibiotic treatments. Complicated UTIs cause upper UTIs, they require a longer duration of antibiotic course, and they show a higher rate of treatment failure and recurrent infections (4). UTIs can be community, healthcare-associated, or auto-infected. Community-acquired infection occurs because of poor hygiene whereas self–infected infection occurs mostly in immune-compromised patients (5, 6). Hospital-acquired UTIs occur after 48 hours of hospitalization and community-acquired UTIs occur after less than 48 hours of hospitalization. The frequency of UTIs varies due to various factors such as age, gender, use of catheters, and prolonged use of antibiotics

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(7, 8). Gram-positive and gram-negative bacteria are involved in UTIs, such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus (S. aureus), Enterococcus faecalis (E. faecalis), Proteus mirabilis (P. mirabilis)* and some fungi such as *Candida albicans* (9). As compared to males, females have more chances of getting UTIs because of a shorter urethra, and during menopause, females suffer from lots of hormonal changes, such as a drop in estrogen levels. Because of that, urethral skin becomes thinner, and it reduces the colonization of the bacteria lactobacillus, which reduces vaginal pH by producing acid which protects against infections (10). During pregnancy, women are more prone to get UTIs due to impaired immunity, and other risk factors include immune-compromised patients, people who are suffering from diabetic mellitus, renal stones, and the use of catheters, contraceptives, and intrauterine devices (11).

Nowadays, a wide range of antimicrobial agents are available in the market. These products are intended to either eradicate or prevent the development of pathogenic microorganisms, thereby aiding the management of infectious diseases. However, a serious problem emerges, as many of these bacteria become multi-drug resistance (MDR) strains, as a result of their increasing resistance to these drugs. Certain gram-negative bacteria have developed resistance to nearly all currently available antibiotics, resulting in the emergence of phenotypes that are highly or entirely resistant. This situation is often referred to as the 'antibiotic resistance crisis' (12).

Over the last few decades, there has been an increase in MDR day by day, causing serious health problems. Earlier, MDR bacteria were restricted to only nosocomial acquired infections, but now certain MDR bacteria are common sources of community-acquired infections (13). Gram-positive and gram-negative bacteria exhibit an elevated prevalence of MDR, which include some gram-negative bacteria such as *E. coli, K. pneumoniae*, *Acinetobacter baumannii (A. baumannii), P. aeruginosa* including gram-positive bacteria *S. aureus, S. pneumoniae, E. faecalis* and *E. faecium* (12). Healthcare professionals face numerous challenges when treating infections caused by MDR pathogens, and immune-compromised patients are more vulnerable (14). MDR bacteria develop resistance to various classes of antibiotics by successfully transferring the resistance gene among these bacterial species. Certain bacteria develop unique defense mechanisms against antibiotics, such as efflux pump, decreased permeability to lipopolysaccharide layer, target modification, and releasing degrading enzymes. MDR bacterial infections are commonly associated with a worse prognosis (15). This group included various drug-resistance strains such as *Acinetobacter, Campylobacter*, fluconazole-resistant *Candida* (a fungus), ESBL-producing *Enterobacteriaceae*, vancomycin-resistant *Enterococcus* (VRE), *P. aeruginosa,* non-typhoidal *Salmonella, Salmonella typhi*, *Shigella*, Methicillin resistance *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae,* and *Sternoclavicular tuberculosis* (16).

Worldwide, the most common drug used to treat bacterial infection is a beta-lactamase antibiotic, which is the main factor for developing resistance in gram-negative bacteria. The constant exposure of bacterial strains to the various classes of beta-lactam antibiotics results in the mutation and continuous synthesis of beta-lactamases in these bacteria, which leads to increases in their efficacy against the newly formed antibiotics (17). These enzymes are commonly known as extended-spectrum beta-lactamase (ESBLs). Bacteria producing ESBL can hydrolyze β lactam antibiotics such as penicillin, cephalosporin, and monobactams but not cephamycins and carbapenems (18). In addition to ESBL, producers are also resistant to other classes of antibiotics, such as aminoglycosides, chloramphenicol, sulfonamides, and tetracyclines (19). The ESBL-producing bacteria is the main cause of nosocomial illness and increasing drug resistance in both hospitals and the community. ESBL-producing organisms increase hospitalization time, lower cure rates, and increase morbidity and mortality rates (20). ESBL-producing bacteria have been increasing exceedingly over the past decades because the plasmid which is encoding for ESBLs is easily transmittable between species, especially by bacterial conjugation. The most common ESBL-producing gram-negative bacteria are *E. coli* and *K. pneumoniae*. Other gram-negative bacteria producing ESBL enzymes are *P. aeruginosa, Acinetobacter spp., Enterobacter aerogenes, Citrobacter koseri, Citrobacter freundii,*

Enterobacter cloacae, Serratia marcescens, Morganella morganii, Providencia spp., Burkholderia cepacia, Alcaligenes fecal. Most nosocomial cases of UTIs are due to ESBL-producing *K. pneumoniae*, and ESBL-producing *E. coli* is the most common etiological agent for community-acquired UTIs. Infections caused by other ESBL-producing Enterobacteriaceae are respiratory tract infections, intra-abdominal infections, and bacteremia (21). Common ESBL genes encoding for enzymes are TEM, SHV, CTX-M, and some other types are OXA and some uncommon types BEL-1, PER, BES-1, VEB, and SFO-1. Most of the ESBL producers are either SHV or TEM types, and they are associated with nosocomial infections, while CTX-M-producing isolates are mostly associated with community-acquired infections, and they confer resistance to various classes of antibiotics such as aminoglycosides, cotrimoxazole and fluoroquinolones (22). These genes contribute to the spread of antibiotic resistance. Biofilm-related MDR UTIs present a significant clinical challenge. It is a part of the bacterial survival mechanism. Biofilm-producing bacteria have certain advantages like quorum sensing, increased tolerance to the host immune system, increased bacterial conjugation, and conferred resistance to various classes of antibiotics; this is bacteria's more effective way to survive in the presence of antibiotics (23). To form biofilm, microbial cell attaches themselves to solid surfaces or to each other and enclose themselves in a polymeric matrix or slime layer, which is a sticky type of material. The matrix of biofilm-producing bacteria consists of polysaccharides, proteins, and extracellular DNAs. Biofilm-producing bacteria are involved in chronic and persistent infections make them resistant to harsh environmental conditions and provide protection from both innate and acquired immunity of the host immune system, which leads to a high rate of morbidity and mortality (24). In device-associated nosocomial infection, bacteria form biofilm on various medical devices, causing chronic and life-threatening conditions (25).

However, a serious problem arises when these bacteria develop resistance to these drugs, with many bacteria becoming multidrug-resistant strains. Although these drugs eradicate the infection successfully, they have several side effects. As a result, there is increasing demand for natural sources as antimicrobial agents, which are safer, have no side effects, and are cost-effective. People are favouring natural sources as alternative medication to control MDR infections and to ensure the safe administration of medications. In contrast to conventional antibiotics, plants present a safe, natural, affordable, and well-researched alternative supply of antibiotics.

Methodology

Plant Leaf Collection and Extract Preparation

The reported plants were collected from villages in the Jatani locality of Khordha District, with species identification authenticated by experts at the Regional Plant Resource Centre (RPRC) Bhubaneswar. To gather traditional knowledge, interviews were conducted with approximately 60 respondents across 30 hamlets, using the snowball sampling method. The collected information was systematically recorded and documented (Table 1). Crude extracts were obtained using a bioassay-guided extraction process with Soxhlet apparatus, utilizing both polar (methanol) and non-polar (n-hexane) solvents. Both the solvent extracts of dried leaf samples dissolved in 10% v/v dimethyl sulfoxide (DMSO) were used for this study, as detailed (26).

Table 1: Ethnomedicinal Use of Three Medicinal Plants

Sl. No.	Plant name, Family name	Parts used	Ethnomedicinal uses
1	*Psidium guajava* L.; Myrtaceae	*Leaf/Bark*	Leaf and bark are used for cough, diarrhoea, dysentery, vomiting, oral ulcers, certain respiratory and gastrointestinal disorders, gum wounds, ulcers, pain, platelet enhancement, and blood pressure.

2	*Syzygium cumini* L.; Myrtaceae	Leaf/ Bark/ Seed	It is used for sore throat, bronchitis, asthma, dysentery, ulcers, blood purifiers, and various metabolic disorders, including blood glucose regulation or antidiabetic regimens have been treated with this.
3	*Punica grantum* L.; Lythraceae	Leaf/ Bark/ Peel	It is used for the treatment of ulcers, diarrhoea, dysentery, male infertility, coughs, sore throats, urinary infections, digestive disorders, arthritis, tapeworms, and skin diseases with great nutritional value.

Preliminary Phytochemical Screening

The qualitative analysis of phytochemical constituents of selected 3 medicinal plants was conducted to assess the presence of alkaloids, steroids, flavonoids, glycosides, tannins, and saponins, and the results were summarized in Table 2 (26).

Isolation, Identification of Uropathogenic Bacterial Strains, and Antibiotic Sensitivity Test

In this study, one Gram-positive bacteria, *S. aureus*, along with three Gram-negative bacteria, *E. coli, K. pneumoniae*, and *P. aeruginosa*, were used. All the bacterial strains were directly isolated from urine samples of the UTI patients attending IMS and Sum Hospital, Bhubaneswar. Identification of the bacterial strains was done based on their colony morphology and biochemical tests. Antibiotic sensitivity testing for all bacterial strains was performed using Kirby-Bauer's disc diffusion method on a 4 mm thick Mueller–Hinton agar (MHA) Plate, following standard antibiotic susceptibility guidelines (27).

Test for ESBL Detection and Biofilm Formation

The test strains were cultured overnight and adjusted to 0.5 McFarland standard, and by using MHA, a lawn culture was prepared. Antibiotic discs containing ceftazidime and ceftazidime combined with clavulanic acid were placed 20 mm apart on the plate, which was then incubated at 37ºc overnight. Then the diameter of the zone of inhibition was measured to detect ESBL producers. An increase in zone diameter ≥5 mm for ceftazidime/ clavulanic acid disc compared to ceftazidime disc was used as the criterion for identifying an ESBL producer (28). The Congo Red Agar was utilized to detect the production of biofilm. Tested isolates were streaked on a Congo Red Agar plate and incubated at 37º c for 24 hours. The development of black colour colonies suggested the biofilm production (29) (Figure 1).

Figure 1: Biofilm Detection in Congo Red Agar Medium Showing Black Colour Colonies is Positive for Biofilm Production

Antibacterial Activity Testing of Plant Extracts

The isolated bacterial strains demonstrated resistance to multiple commonly used antibiotics. Antibacterial efficacy of methanol and n-hexane extracts from selected plants was assessed using the agar well diffusion method, with amikacin at 50 µg/ml serving as the reference standard. The antibacterial activity was evaluated, and the results of the third repetition were presented (27).

Determination of MIC and MBC of Plant Extracts

Stock solutions of the methanol leaf extracts were prepared, using a concentration of 50 mg of plant extract per ml in a 10% DMSO solution with distilled water. These stock solutions were then serially diluted to achieve final concentrations of 1.56, 3.12, 6.25, 12.5, 25, and 50 µg/mL in the DMSO solution. The minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of the methanol leaf extracts were determined using a 96-well microtiter plate.

Statistical Analysis

All the data is stored using the Windows Excel tool for creating a list of random sampling frames. IBM-SPSS statistics software version 20 was used to analyse the collected data. Indeed, frequencies, means, and standard deviations were used to construct descriptive data. Calculations and tabulations of ratios and percentages were made.

Results

Preliminary Phytochemical Screening

The below-mentioned results (Table 2) indicated that saponin and steroids will get solubilized in n-hexane as appeared in corresponding leaf extracts, where flavonoids and alkaloids class of constituents were observed in methanol extracts. Out of 4 isolated organisms (*E. coli, K. Pneumoniae, P. aeruginosa*, and *S. aureus*), three gram-negative organisms were found to be ESBL producers, except for *S. aureus*. The antibiotic resistance patterns of each pathogenic bacterium were assessed using specific antibiotic discs (as detailed in Table 3).

Table 2: Qualitative Analysis of Phytochemical Constituents

	Psidium guajava L.		***Syzygium cumin L.***		***Punica granatum L.***	
Phytochemicals	**Methanol extracts**	**n-Hexane extracts**	**Methanol extracts**	**n-Hexane extracts**	**Methanol extracts**	**n-Hexane extracts**
Alkaloids (Mayer's test)	+	-	+	-	+	-
Steroids (Salkowski test)	-	-	-	-	-	+
Flavonoids (Sodium hydroxide test)	+	-	+	-	+	-
Saponins (Foam test)	+	+	+	+	-	+
Tannins (Ferric chloride test)	+	+	+	+	+	+
Glycosides (Borntrager's test)	+	-	+	+	+	-

Table 3: Bacterial Susceptibility Testing to Antibiotics

Bacterium	AC	GM	CZA	AK	TZP	CF	OF	NX	Va	CRO	LZ	CIP	ETP	FEP
E. coli	R	MS	R	MS	S	MS	R	R	ND	R	MS	R	S	R
K. pneumoniae	R	R	R	S	MS	R	MS	R	MS	R	S	MS	S	R
P. aeruginosa	R	MS	R	R	MS	R	R	R	ND	R	MS	R	ND	R
S. aureus	MS	MS	ND	MS	S	MS	MS	R	MS	MS	S	R	ND	ND

Note: Antibiotics, Ac: Amoxicillin- clavulanate, AK: Amikacin, CF: cefpodoxime, CIP: Ciprofloxacin, CRO: Ceftriaxone, CZA: Ceftazidime, ETP: Ertapenem, FEP: Cefepime, GM: Gentamicin, LZ: Linezolid, NX: Norfloxacin, OF: Ofloxacin, TZP: Tazobactam, Va: Vancomycin

Here, Figure 2 illustrates the percentage of resistance of four bacterial isolates- *E. coli, K. pneumoniae*, *P. aeruginosa*, and *S. aureus*- to a range of antibiotics. For most antibiotics, resistance levels were high across all four bacteria, with a percentage approaching 100 % in several cases, particularly for antibiotics such as AC, CF, NX, CRO, and CIP. However, resistance levels vary by species for certain antibiotics. For instance, *S. aureus* exhibited lower resistance to GM and AK compared to the other bacteria, while *E. coli* showed lower resistance to ETP and CF. A few antibiotics, such as LZ, displayed relatively low resistance across most species, except for K. pneumonia. Overall, the data underscores the widespread issue of antibiotic resistance, suggesting challenges in effectively treating infections caused by these pathogens due to high resistance rates across many commonly used antibiotics.

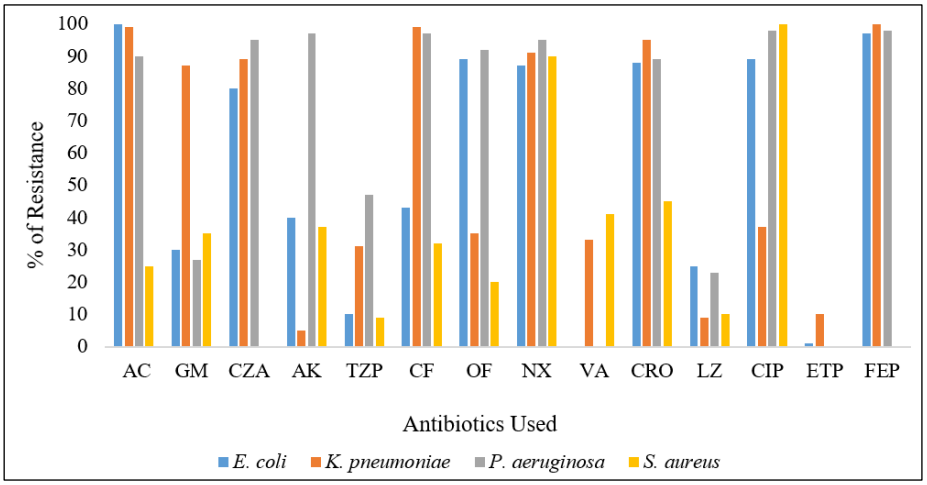

Figure 2: Antibiotic Resistance Percentage of Isolated Organisms (In the X Axis Antibiotics are Mentioned as Follows: Ac: Amoxicillin- Clavulanate, GM: Gentamicin, CZA: Ceftazidime, AK: Amikacin, TZP: Tazobactam, CF: Cefpodoxime, OF: Ofloxacin, NX: Norfloxacin, Va: Vancomycin, CRO: Ceftriaxone, LZ: Linezolid, CIP: Ciprofloxacin, ETP: Ertapenem, FEP: Cefepime. In the Y-Axis, the Percentage of Resistance is Mentioned)

Antibacterial Activity of Plant Extract

P. guajava methanol extract showed moderate inhibition against all bacteria, with the highest inhibition zone for *K. pneumoniae* (16± 0.78 mm) and the lowest for *E. coli* (11 ± 3.82 mm). The n-hexane extract of this plant exhibited the least activity, especially against *S. aureus* (8± 3.24 mm). *S. cumini* extracts displayed stronger antibacterial activity, particularly the methanol extract, which was most effective against *P. aeruginosa* (21±1.22 mm) and *K. pneumoniae* (20± 0.46 mm). The n-hexane extract of *S. cumini* also showed notable activity, particularly against *E. coli* (16 ± 3.22 mm). Methanol extract of *P. granatum* demonstrated the lowest inhibition against *E. coli* (10± 1.22 mm) and *S. aureus* (14± 0.64 mm), with *K. pneumoniae* (18± 0.86 mm) showing better inhibition. Similarly, the n-hexane extract showed relatively lower inhibition across all strains, with the highest against *K. pneumoniae* (13± 2.36 mm). Amikacin was used as a positive control, while DMSO was used as the negative control in this study (Table 4).

Table 4: Antibacterial Efficacy of Crude Extracts of Three Selected Plants

Used bacterium strains for the study	*P. guajava*		*S. cumini*		*P. granatum*		Amikacin (50µg/mL) as positive control	DMSO as negative control
	Methanol extract	n- hexane extracts	Methanol extract	n- hexane extracts	Methanol extract	n- hexane extracts		
E. coli	11±3.82	09±3.24	18±2.82	16±3.22	10±1.22	08±2.20	22±0.00	------
K. pneumonia	16±0.78	14±3.24	20±0.46	18±2.22	18±0.86	13±2.36	26±0.00	------
P. aeruginosa	15±0.64	14±0.80	21±1.22	15±0.42	17±1.20	10±1.90	25±0.00	------
S. aureus	14±2.35	08±3.24	16±3.02	13±0.76	14±0.64	08±2.50	24±0.00	------

Recorded antibacterial efficacy information of zone of inhibition (mm) of crude extracts of three selected plant leaves derived from two different solvent systems using agar well diffusion assay at 25 µg/ mL.

The results of the study imply that these plant extracts have bioactive substances that may act as organic antibacterial agents. Among all the strains studied, *S. cumini* extracts showed the most antibacterial activity, particularly against *P. aeruginosa* and *E. coli*.

MIC and MBC Values

E. coli exhibited the highest MIC for *P. guajava* (50 ± 0.34 µg/mL) and *P. granatum* (50± 0.34 µg/mL), while *S. cumini* had the lowest MIC (25 ± 0.33 µg/mL). All plant extracts showed lower MICs against K. *pneumoniae and P. aeruginosa*, particularly with *S. cumini* (12.5± 0.74 µg/mL). The MBC values for *P. guajava* were greater than 100 µg/mL for *E. coli* and *S. aureus*, indicating a higher concentration is required for bactericidal effect. S. aureus showed significant susceptibility with a MIC of 25 ± 0.33 µg/mL for *S. cumini*. Comparatively, Amikacin the positive control, exhibited consistent MIC and MBC values at 50± 0.00 µg/mL, whereas the negative control (DMSO) showed no inhibition. Among all examined microorganisms, *S. cumini* consistently had the lowest MICs and MBCs, demonstrating potent antibacterial action, particularly at doses of 12.5 and 25 µg/mL. Both *P. granatum* and *P. guajava* had antibacterial action, however they usually needed larger doses (50 and 100 µg/mL) to get comparable MIC and MBC values. The MIC values for each of the three plant extracts were found to be lower than the MBC values (Table 5), suggesting that larger doses are required to eradicate the bacteria entirely as opposed to merely stopping their growth. The effectiveness of the plant extracts is arranged according to the decreasing order, *S. cumini* > *P. granatum* > *P. guajava.*

Table 5: MIC and MBC Values of Methanol Crude Extracts of Three Selected Plants

Used bacterium strains for the study	*P. guajava*		*S. cumini*		*P. granatum*		Amikacin (50µg/mL) as positive control	DMSO as negative control
	MIC	MBC	MIC	MBC	MIC	MBC		
E. coli	50±0.34	>100±0.54	25±0.33	50±0.54	50±1.22	>100±0.86	50±0.00	------
K. pneumonia	25±1.24	50±4.12	12.5±0.74	25±0.12	25±0.86	50±1.36	25±0.00	------
P. aeruginosa	25±2.38	50±6.20	12.6±0.10	25±2.20	25±2.20	50±3.90	25±0.00	------
S. aureus	50±1.26	100±0.26	25±0.33	50±0.00	50±0.86	100±2.50	50±0.00	------

Recorded antibacterial efficacy of methanol crude extracts in the form of MIC and MBC of three selected plant leaves using 96 microtiters well plate assay taking different conc. 12.5, 25, 50 and 100 µg/mL

Discussion

The antibacterial activity presented herein validated the ethnic information of the curative effect of the used plants in bacterial infections. Additionally, *P. guajava, S. cumini*, and *P. granatum* are edible plants, and their parts are used to treat UTI, suggesting the absence of host toxicity, a fact corroborated by this work. Moreover, secondary metabolites of these plants are expected to have contributed to the recorded effective antibacterial activity. The presence of a higher number of phenolic classes of phytocompounds in the above leaf extracts confirms their higher antibacterial potency and lower toxicity profiles (30). The Molecular docking results of the reported compounds of these plants suggested that the presence of bioactive compounds like ursolic acid, epifriedelanol, and punicacortein synergistically enhanced the crude extracts' antibacterial activity (30). In literature, leaf extracts from *Symplocos racemosa* and several other plants like *Anogeissus acuminata, Boerhaavia diffusa, Soymida febrifuga, Terminalia chebula, tinospora cordifolia, Tribulus terrestris* had shown effective control capacity against MDR bacteria (26, 31).

Nowadays, infection caused by MDR microbes is widespread and creates an important treatment challenge across the globe. Notably, organisms that produce antibiotic resistance carry genes for antibiotic resistance in both plasmids and chromosomes, and their transfer mechanisms remain functional. Consequently, susceptible bacteria can acquire these genes and/or transposons horizontally through processes such as bacterial transformation or conjugation (27). UTIs caused by MRSA, ESBL, VRE, and Carbapenem resistance organisms are involved in higher morbidity and mortality. As MDR microbes confer resistance to almost all currently available antibiotics, which creates a threat in the health sector, treating an infection caused by an MDR organism involves high treatment failure, increased treatment costs, and longer hospital stays (32).

It was seen that 4 bacteria isolated from urine samples were resistant to the following class of antibiotics: aminoglycosides, β-lactams, and cephalosporins signifying most bacterial strains as resistant to most antibiotics. However, certain plants like *S. cumini*, *P. guajava*, and *P. granatum*. exhibited significant control over all 4 selected uropathogenic MDR bacterial strains. Pathogens susceptible to antibiotics exhibit restricted virulence due to the effectiveness of the antibiotic in controlling their growth within a living organism. The current method of using methanol for phytochemical extraction is distinctive, as this solvent effectively extracts a wide range of phytocompounds, from polar to non-polar (26). Antibiotics have several modes of action to combat bacterial actions. The complementary use of phytocompounds alongside mainstream antibiotics has gained attention, especially considering the numerous mechanisms of drug resistance. This approach leverages the effectiveness of phytocompounds as non-microbial antimicrobials (33-35).

A study reported from Nigeria, both aqueous and ethanol extract of *P. guajava* was found to be impactful against *S. aureus* strains (36) but the zone of inhibition was less as compared to the current study, methanol extract of *P. guajava* was found to be more impactful against selected pathogenic bacteria. In this study, the methanol extract of *S. cumini* was found to be more effective against the n-hexane extract of *S. cumini* and showed greater activity against *P. aeruginosa.* However, a separate study reported that the methanol extract of *S. cumini* exhibited no activity against *P. aeruginosa* (37). In contrast to the current study, previous research reported the highest zone of inhibition as 16mm against *K. pneumonia* and *S. aureus.* In the present study, however, the zone of inhibition recorded against *K. pneumonia* was 20±0.46 mm, which is higher, while the zone inhibition for *S. aureus* was found to be 16±3.02 mm, closely aligning with the earlier findings (37). A study carried out in Coimbatore documented that both the methanol and aqueous extract of *S. cumini* were effective against pathogenic microorganisms, with a zone of inhibitions recorded as 6 mm for *E. coli,* 7 mm for *P. aeruginosa* and 8 mm for *S. aureus*. These zones of inhibitions are notably smaller as compared to the current study (38). In the current study, the methanol extract of *P. granatum* was found to be

more effective against *K. Pneumonia* compared to the other tested organisms.

Conclusion

The antibiograms of four pathogenic bacterial isolates against 14 antibiotics demonstrated multidrug resistance across all strains. In response, three selected plants, *S. cumini, P. guajava, and P. granatum,* showed promising antibacterial activity, often displaying lower MIC and MBC values. All plants used in this study possess established ethnomedicinal properties, suggesting potential as complementary medicines. The promising activity of *S. cumini* indicates the need for further studies focused on isolating pure bioactive compounds to enhance the arsenal against MDR bacteria. This work supports the potential of these plants as effective sources of plant-based antimicrobials targeting MDR uropathogens.

Abbreviations

E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, K. pneumoniae: Klebsiella pneumonia, MBC: Minimum Bactericidal Concentration, MDR: Multidrug resistance, MIC: Minimum Inhibitory Concentration, MRSA: Methicillin resistance *Staphylococcus aureus, P. granatum*: *Punica grantum, P. guajava: Psidium guajava, P. mirabilis: Proteus mirabilis, S. aureus: Staphylococcus aureus, S. cumini: Syzygium cumini,* UTI: Urinary Tract Infection, VRE: Vancomycin resistance *Enterococcus.*

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Author Contributions

Susmita Chakrabarty: Collected, analysed and interpreted the data, and involved in drafting the manuscript. Mamalisa Sahoo: interpreted the data and involved in drafting the manuscript. Monali Priyadarsini Mishra: Designed the study and involved in drafting the manuscript, Dattatreya Kar: Designed the study and involved in drafting the manuscript. All authors read the manuscript and approved the final copy for submission.

Conflict of Interest

Authors declare no conflict of interest.

Ethics Approval

Not applicable.

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