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Original Article

Antibacterial Activity of Selected Fruit Juices against Multidrug-Resistant Bacterial Pathogens Involved in Urinary Tract and Sexually Transmitted Infections among Tribal Women in Madhya Pradesh, India

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Objectives: The aim of this study was to evaluate the effect of fruit juices on Multi-Drug Resistant (MDR) bacterial pathogens involved in Urinary Tract Infections (UTIs) and Sexually Transmitted Infections (STIs) among tribal women in the district Anuppur, Madhya Pradesh, India.

Methods: Fresh juices of lemon (*Citrus limon*), amla/Indian gooseberry (*Phyllanthus emblica*), pineapple (*Ananas comosus*), mosambi/sweet lime (*Citrus limetta*), orange (*Citrus sinensis*), kiwi (*Actinidia deliciosa*), and pomegranate (*Punica granatum*) fruits were evaluated for in vitro antibacterial activity against bacterial pathogens involved in UITs and STIs among tribal women. Physico-chemical analysis of fresh fruits was also carried out by measuring the pH, moisture, protein, fat, crude fibre, carbohydrate, and ascorbic acid content.

Results: Lemon and amla juice showed better antibacterial activity against the pathogens as compared to other juices. MIC results fruit juices against UTIs and STIs pathogens vary depending on the specific pathogen and juice chemical constituents. The physico-chemical analysis showed that the moisture content was highest in mosambi (90%), followed by orange (87%). Ascorbic acid content was found highest in amla (540 mg/100 g), followed by kiwi (90.3 mg/100 g). Pomegranate showed highest concentration of carbohydrate (15.28 g/100 g), fat (1.28 g/100 g), and protein (1.65 g/100 g). Lemon juice had lowest pH of 2.20, followed by amla 2.67.

Conclusion: The lemon juice showed highest antibacterial activity against MDR bacterial pathogens involved in UTIs and STIs among tribal women in district Anuppur, Madhya Pradesh, India. The low pH of lemon may be responsible for its high antibacterial activity as compared to other juices.

Keywords: uropathogens, sexually transmitted infection, multi-drug resistant, antibacterial activity, fruit juices

INTRODUCTION

Urinary tract infections (UTIs) and sexually transmitted infections (STIs) commonly affect women. UTIs are caused by bacteria entering the urinary tract through the urethra, while STIs are transmitted through sexual contact and can be caused by viruses, bacteria, or parasites. Despite efforts to address these infections, their incidences continue to rise, negatively impacting women's quality of life [1] and contributing to maternal morbidity, ectopic pregnancies, abortions, low birth weights, infant mortality, and infertility [2]. Prompt and accurate diagnosis and treatment are crucial for effective management of these infections [3]. However, there is a lack of systematic studies on the diagnosis and treatment of UTIs and STIs in India [4-

6]. Studies indicate a significant prevalence of UTIs and STIs among tribal women in India [7], and these conditions impose a substantial burden on tribal women in the Anuppur district of Madhya Pradesh. The high prevalence of UTIs can be attributed to factors such as poor hygiene, limited education, and reduced access to quality healthcare services [8].

The indiscriminate use of antibiotics has led to the emergence of drug-resistant microbes, posing challenges in the treatment of infections and resulting in longer illness periods, increased hospitalizations, and higher healthcare costs. Antimicrobial resistance is a global health crisis and is projected to surpass cancer as the leading cause of mortality by 2050 [9]. Inadequate diagnostic methods often lead to the inappropriate use of antibiotics, contributing to the development of antibiotic resistance [10], which can emerge and spread globally within a short period after the introduction of new antibiotics [11]. Multi-drug resistant (MDR) microorganisms, which are not susceptible to multiple classes of antimicrobials, pose a significant global health threat [12].

Antibiotic resistance has necessitated the search for novel, safer, and cost-effective antimicrobial agents, especially those of herbal origin. Plants are a useful source of medicines because they contain bioactive components that have specific physiological impacts on human health [13]. Plants offer a rich source of bioactive compounds, such as tannins, terpenoids, flavonoids, and alkaloids, which possess antibacterial properties [14]. These bioactive components can have bactericidal or bacteriostatic effects on MDR pathogens and serve as potential precursors for developing new antibiotics [15]. Therefore, plant-based antimicrobial agents present a promising and accessible therapeutic option for treating infections caused by MDR strains [16], offering new hope for combating challenges posed by increasing antibiotic resistance [17]. Various studies have reported the antimicrobial potential of medicinal plants in the treatment of UTIs and STIs, including reports from Bangladesh, Uganda, Nigeria, and South Africa [18-21].

The present study was carried out to assess the antibacterial activity of the fresh juices of lemon (*Citrus limon*), amla/Indian gooseberry (*Phyllanthus emblica*), pineapple (*Ananas comosus*), mosambi/sweet lime (*Citrus limetta*), orange (*Citrus sinensis*), kiwi (*Actinidia deliciosa*), and pomegranate (*Punica granatum*) fruits against MDR strains of bacteria responsible for UTI and STI infections.

MATERIALS AND METHODS

1. UTI and STI pathogens

This study was carried out in the Infection Biology and Molecular Reproductive Toxicology Lab, Department of Zoology, IGNTU, Amarkantak, Madhya Pradesh, India. In a previous study, 550 urine samples were collected from tribal women suffering from UTIs in the district of Anuppur. Bacterial strains were isolated, identified, and cryopreserved from 360 culture-positive samples [8], and antibiotic sensitivity tests were performed on their major bacterial isolates. For the present study, vaginal swab samples were collected from 181 tribal women symptomatic for STIs. This study was approved by the institutional ethics committee (Ref. No. IGNTU/IEC/01/2019) and Birsa Munda Government Medical College, Shahdol (Ref. No. IERC/22/06/001).

2. Preparation of fruit juices

Fresh, ripened fruits of lemon, amla, pineapple, mosambi, orange, kiwi, and pomegranate were purchased from the local market of Pushprajgarh block in district Anuppur, Madhya Pradesh, India. The fruits were cleaned under running tap water, surface disinfected with 70% alcohol, and rinsed thoroughly with sterile distilled water. The fruits were chopped into small pieces and ground into a paste using a mortar and pestle. Juices were obtained by squeezing the paste using a sterile muslin cloth. The juice was filtered using Whatman filter paper no. 40 and immediately stored at 4℃ for later use in antibacterial assays.

3. Physicochemical analysis of fresh fruits

Physicochemical analyses of the fresh fruits were conducted. The pH was estimated using a Cyber Scan 2100 pH/MV/ION/benchtop pH meter. The moisture content was evaluated by drying 100 mL of each fruit juice sample in an oven at 105°C until the weight remained constant. The protein content was determined using the Kjeldahl apparatus and calculated using a conversion factor (nitrogen content 6. 25) [22]. Total fat was estimated by the AOAC 2012 method using an automatic Soxhlet apparatus with ether as the solvent [23]. Crude fiber was also calculated using the AOAC 2012 method [23]. The carbohydrate content was determined by subtracting 100 from the total

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of the percentages of moisture, ash, protein, fat, and crude fiber (%Carbohydrate = 100 − (%moisture + %ash + %protein + %fat + %crude fiber) [22]. Ascorbic acid content was measured using the 2, 6-dichloroindophenol titrimetric method [23].

4. Antibacterial activity

The antibiotic sensitivity tests (ASTs) on the major bacterial isolates were performed using the disk diffusion method as described previously [24]. Antibiotic disks were purchased from Himedia (Mumbai, India). The bacteria isolated from UTI patients were subjected to AST using the following antibiotics: nitrofurantoin (NIT), nalidixic acid (NA), carbenicillin (CB), gentamicin (GEN), ampicillin (AMP), co-trimoxazole (COT), sulfamethoxazole (SM), penicillin-G (P), and tetracycline (TE). For the STI clinical isolates, the following antibiotics were used: AST was performed with cefixime (CFM), ceftriaxone (CTR), ciprofloxacin (CIP), levofloxacin (LE), ofloxacin (OF), norfloxacin (NX), tetracycline (TE), doxycycline (DO), roxithromycin (RO), azithromycin (AZM), and erythromycin (E). Determining the antibacterial activity of the fruit juices was conducted using the agar well-diffusion method on Muller Hinton Agar (MHA), according to the previously standardized protocol in our laboratory [14]. Briefly, 100 μL freshly revived, 0.5 McFarland's bacterial suspension was plated on MHA and spread uniformly. Wells were created using a sterile cork borer. The fruit juices (100 μL) were poured into the wells for initial screening for antibacterial activity. The plates were left in a laminar air flow chamber for 10 minutes to allow the juice to diffuse before being incubated for 24 hours at 37°C. The zone of bacterial growth inhibition was measured using the HiAntibiotic Zone Scale.

5. Minimum inhibitory concentration (MIC)

The MIC of fruit juices with promising antibacterial activity was assessed. The antibacterial fruit juices (100%) were diluted with sterile distilled water to obtain 80%, 60%, 40%, and 20% concentrations. Sterile distilled water was used as a solvent control. The lowest concentration of juice visibly inhibiting growth on the agar plate after 24 hours was recorded as the MIC [22]. All experiments were performed in triplicate.

RESULTS

The physicochemical analysis of fresh fruit was conducted, and the results are presented in Table 1. Mosambi showed the highest moisture content (91%), followed by orange (87%), pineapple (86%), amla (84%), lemon (82%), kiwi (82%), and pomegranate (77%). The total protein contents in amla, pomegranate, and kiwi were 1-2 g/100 g, whereas in orange, pineapple, mosambi, and lemon, it ranged from 0.5-1 g/100 g. All fruits had fat contents below 1 g/100 g, except for pomegranate, which had a fat level of 1.28 g/100 g. The highest content of crude fiber was found in lemon (8.81 g/100 g), followed by pomegranate (3.80 g/100 g), kiwi (3.42 g/100 g), orange (2.78 g/100 g), mosambi (2.7 g/100 g), and pineapple (1.23 g/100 g). Carbohydrate contents varied considerably among the fruits. Pomegranate (15.28 g/100 g) had a considerably higher carbohydrate content than pineapple (11.72 g/100 g), kiwi (11.65 g/100 g), orange (8.13 g/100 g), lemon (7.25 g/100 g), and mosambi (4.85 g/100 g). The ascorbic acid content was highest in amla (540 mg/100 g) followed by kiwi (90.3 mg/100 g) and mosambi (54.8 mg/100 g). Pomegranate had the lowest ascorbic acid content (12.15 mg/100 g).

The results of bacterial growth on selective media and the

Table 1. Physico-chemical analysis of fresh fruits

Fruit	pH	Moisture (%)	Physico-chemical characteristics of fruits per 100 g				
			Ascorbic acid (mg)	Protein (g)	Fat (g)	Crude fibre (g)	Carbohydrate (g)
Amla	2.67	84	540	1.5	0.30	4.61	6.94
Pomegranate	3.17	77	12.15	1.65	1.28	3.80	15.28
Kiwi	3.15	82	90.3	1.21	0.67	3.42	11.65
Orange	3.10	87	49.2	0.83	0.17	2.78	8.13
Pineapple	2.76	86	20.6	0.58	0.29	1.23	11.72
Lemon	2.20	82	52	0.98	0.32	8.81	7.25
Mosambi	3.08	91	54.8	0.77	0.21	2.70	4.80

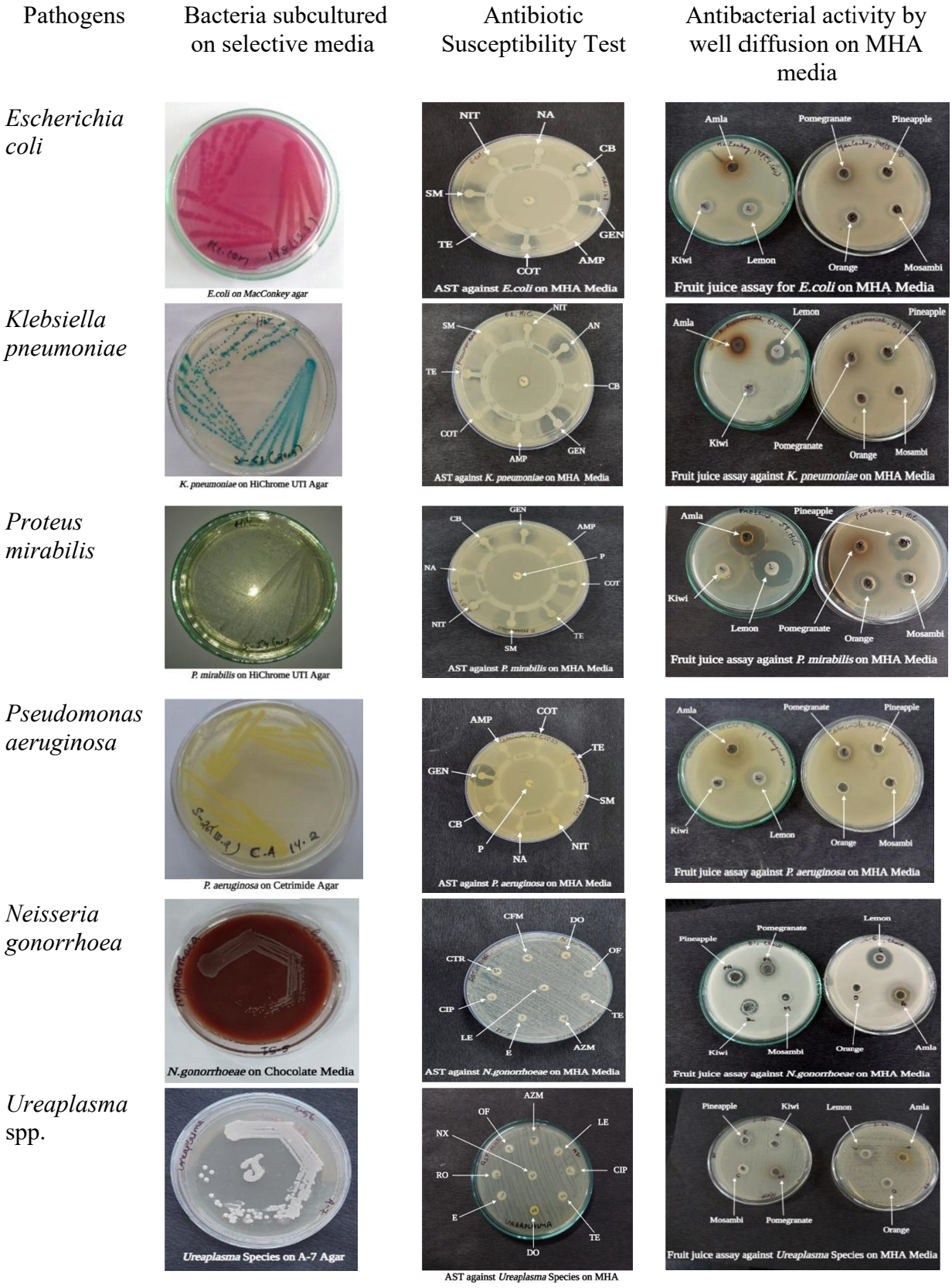

Figure 1. Growth of UTI and STI pathogens on specific culture media, AST, and antibacterial activity of fruits juices.

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Table 2. Antibiotic sensitivity profile of *E. coli* isolated from UTI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-7	CB, COT, P, GEN, SM
2.	CI-10	TE, NIT, COT, P
3.	CI-11	NA, TE, P, AMP
4.	CI-25	NA, NIT, P, GEN
5.	CI-27	COT, P, AMP, CB
6.	CI-33	NA, TE, P, NIT, AMP, SM
7.	CI-58	NA, TE, COT, P, SM
8.	CI-59	NA, P, COT, TE
9.	CI-67	TE, COT, P, CB
10.	CI-70	TE, P, AMP, NA
11.	CI-107	COT, P, GEN, SM
12.	CI-118	COT, AMP, TE, P
13.	CI-135	NIT, COT, AMP, GEN
14.	CI-148	NA, CB, NIT, TE, P, AMP

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

Table 3. Antibiotic sensitivity profile of *K. pneumoniae* isolated from UTI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-15	NA, AMP, P, GEN, TE, COT
2.	CI-45	P, TE, COT, NIT
3.	CI-61	NIT, CB, AMP, COT, P, SM
4.	CI-92	NA, AMP, P, NA, NIT, TE, COT
5.	CI-97	AMP, P, GEN, COT
6.	CI-105	NA, AMP, P, NIT, CB
7.	CI-109	NA, AMP, P, GEN, TE, COT
8.	CI-122	NA, AMP, P, COT
9.	CI-129	AMP, P, GEN, COT
10.	CI-156	NA, AMP, P, CB
11.	CI-207	NA, AMP, P, COT
12.	CI-228	P, GEN, NA, COT
13.	CI-295	P, TE, CB, NIT
14.	CI-331	P, SM, NA, TE

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

Table 4. Antibiotic sensitivity profile of *P. mirabilis* isolated from UTI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-24	P, CB, COT, GEN
2.	CI-40	P, AMP, TE, NIT
3.	CI-54	AMP, P, NIT, NA, CB
4.	CI-59	P, SM, TE, NA, GEN
5.	CI-88	NA, P, COT, NIT
6.	CI-91	P, GEN, CB, AMP
7.	CI-112	P, AMP, NIT, NA
8.	CI-132	P, SM, CB, TE, SM
9.	CI-135	P, GEN, COT, NIT
10.	CI-168	P, SM, TE, COT
11.	CI-221	NA, P, GEN, TE
12.	CI-267	P, AMP, S, COT, CB
13.	CI-268	NA, P, TE, NIT
14.	CI-279	P, AMP, SM, NA

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

Table 5. Antibiotic sensitivity profile of *P. aeruginosa* isolated from UTI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-26	COT, TE, SM, NIT, NA, P, CB, AMP
2.	CI-57	NA, P, COT, AMP
3.	CI-82	AMP, P, TE, GEN
4.	CI-141	NA, P, COT, TE, NIT
5.	CI-157	NA, P, SM, GEN
6.	CI-159	GEN, AMP, P, COT, CB
7.	CI-188	NA, P, SM, TE
8.	CI-199	SM, AMP, P, TE, NIT
9.	CI-211	P, SM, CB, NIT
10.	CI-234	P, GEN, CB, COT
11.	CI-255	P, TE, SM, NIT
12.	CI-280	NIT, NA, GEN, P, CB
13.	CI-285	NA, P, TE, NIT

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

antibiotic sensitivity tests are presented in Fig. 1. The samples showing resistance to three or more antibiotics were considered to have MDR. Out of 360 culture-positive urine samples from UTI patients, 55 contained strains with MDR. Of these samples, *Escherichia coli* (Table 2), *Klebsiella pneumoniae* (Table 3), and *Proteus mirabilis* (Table 4) were predominant in 14 samples each, whereas *Pseudomonas aeruginosa* (Table 5) was predominant in 13 samples. Out of 181 culture-positive samples from women with STIs, 37 showed MDR against the tested antibiotics; of these samples, MDR *Neisseria gonorrhoea* (Table 6) and *Ureaplasma* spp. (Table 7) were detected in 20 and 17 samples, respectively.

The antibacterial activity of fruit juices is summarized in

Table 6. Antibiotic sensitivity profile of *N. gonorrhoea* isolated from STI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-11	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
2.	CI-20	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
3.	CI-32	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
4.	CI-34	CFM, CTR, CIP, LE, OF, TE, AZM, E
5.	CI-48	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
6.	CI-51	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
7.	CI-54	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
8.	CI-61	CFM, LE, OF, TE, AZM, E
9.	CI-69	CFM, OF, TE, DO, AZM, E
10.	CI-71	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
11.	CI-73	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
12.	CI-86	CFM, CIP, LE, OF, TE, AZM, E
13.	CI-87	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
14.	CI-90	CFM, CTR, LE, OF, AZM, E
15.	CI-102	CFM, CTR, CIP, LE, OF, TE, E
16.	CI-105	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
17.	CI-106	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
18.	CI-107	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
19.	CI-121	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E
20.	CI-128	CFM, CTR, CIP, LE, OF, TE, DO, AZM, E

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

Table 7. Antibiotic sensitivity profile of *Ureaplasma* spp. Isolated from STI samples

S. no.	Sample ID	Resistance to antibiotics
1.	CI-20	CIP, LE, OF, TE, DO, AZM, E, RO, NX
2.	CI-22	CIP, LE, OF, TE, DO, AZM, E, RO, NX
3.	CI-27	CIP, LE, OF, TE, DO, AZM, E, RO, NX
4.	CI-31	CIP, LE, OF, TE, DO, AZM, E, RO, NX
5.	CI-37	CIP, LE, OF, TE, DO, AZM, E, RO, NX
6.	CI-45	CIP, LE, OF, TE, DO, AZM, E, RO, NX
7.	CI-49	CIP, LE, OF, TE, DO, AZM, E, RO, NX
8.	CI-51	CIP, LE, OF, TE, DO, AZM, E, RO, NX
9.	CI-54	CIP, LE, OF, TE, DO, AZM, E, RO, NX
10.	CI-56	CIP, LE, OF, TE, DO, AZM, E, RO, NX
11.	CI-57	CIP, LE, OF, TE, AZM, E, RO, NX
12.	CI-71	CIP, LE, OF, TE, AZM, E, RO, NX
13.	CI-81	CIP, LE, OF, TE, DO, AZM, E, RO, NX
14.	CI-84	CIP, LE, OF, TE, DO, AZM, E, RO, NX
15.	CI-121	CIP, LE, OF, TE, DO, AZM, E, RO, NX
16.	CI-128	CIP, LE, OF, TE, DO, AZM, E, RO, NX
17.	CI-137	CIP, LE, OF, TE, DO, AZM, E, RO, NX

CI, Clinical Isolates; NIT, nitrofurantoin; CB, carbenicillin; SM, sulfamethoxazole; GEN, gentamicin; AMP, ampicillin; P, penicillin-G; COT, co-trimoxazole; NA, nalidixic acid; CIP, ciprofloxacin; TE, tetracycline; CFM, cefixime; CTR, ceftriaxone; LE, levofloxacin; OF, ofloxacin; DO, doxycycline; AZM, azithromycin; E, erythromycin.

Table 8. Antibacterial activity of fruit juices

Microbial isolates	No. of isolates	Antimicrobial activity of fruits juice (Average zone of inhibition [mm] with SEM)						
		Lemon	Amla	Pineapple	Mosambi	Orange	Kiwi	Pomegranate
E. coli	14	22 ± 0.38	18.36 ± 0.29	17.36 ± 0.29	11 ± 0.45	0	1.43 ± 0	2.21 ± 0.15
K. pneumoniae	14	17.43 ± 0.86	17.21 ± 0.68	15.71 ± 0.73	10 ± 0.54	2.93 ± 0.13	1.43 ± 0	1.5 ± 0.19
P. mirabilis	14	20.07 ± 0.77	18.07 ± 0.71	16.07 ± 1.02	6.29 ± 0.32	3.14 ± 0.22	1.71 ± 0.38	1.79 ± 0.57
P. aeruginosa	13	17.53 ± 0.68	17.77 ± 0.54	17.23 ± 0.7	6.54 ± 0.46	2.31 ± 0	1.61 ± 0.2	2.15 ± 0.4
N. gonorrhoea	20	19.8 ± 0.44	13.55 ± 0.79	10.8 ± 0.49	1.2 ± 0.32	1.25 ± 0.47	1.45 ± 0.16	2.2 ± 0.26
Ureaplasma spp.	17	17.47 ± 0.76	17.23 ± 0.98	15.23 ± 1.05	1.29 ± 0	1.23 ± 0.17	1.35 ± 0.17	1.94 ± 0

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Table 8. Lemon juice showed the highest antibacterial activity against all the MDR isolates, as indicated by the zones of growth inhibition for *E. coli* (22 ± 0.38 mm), *P. mirabilis* (20.07 ± 0.77 mm), *N. gonorrhoea* (19.8 ± 0.44 mm), *P. aeruginosa* (17.53 ± 0.68 mm), *Ureaplasma* spp. (17.47 ± 0.76 mm), and *K. pneumoniae* (17.43 ± 0.86 mm). Good-to-moderate activity was exhibited by amla and pineapple. Kiwi juice had the lowest activity, followed by pomegranate, orange, and mosambi (Table 8).

Table 9 shows the MIC_{50} values of the antibacterial fruit juices against the MDR isolates. The MIC_{50} values indicate the concentrations of fruit juices required to achieve 50% growth inhibition of the test population, measured by zones of inhibition. Lemon was the most effective fruit juice, with an MIC_{50} (zone of inhibition ≤ 11 mm) at 20% concentration for all selected pathogens. Amla also showed good activity with an MIC_{50} at 20% for *E. coli* and *P. aeruginosa* but 40% for *K. pneumoniae*, *P. mirabilis*, *N. gonorrhoea*, and *Ureaplasma* spp. Pineapple showed a MIC_{50} at 60% for most isolates, except for *E. coli*, against which it was also effective at 40%. However, no reduction in MIC_{50} was observed for mosambi against all the selected MDR UTI and STI pathogens (Fig. 2). The MIC_{50}s of fruit juices against UTI and STI pathogens vary by pathogen type and fruit-juice concentration.

DISCUSSION

In the present study, the antibacterial activities of lemon, amla, pineapple, mosambi, orange, kiwi, and pomegranate fruit juices against bacteria involved in UTIs and STIs in women were evaluated. In addition, the ascorbic acid contents, pHs, and physicochemical properties like moisture, protein, fat, crude fiber, and carbohydrate contents of these fruits were evaluated.

The pH of fruit juice plays a crucial role in its sensory quality, as it affects taste, color, and aroma. The pH range for these fruit juices was between 2.20 (lemon) and 3.17 (pomegranate). In fruit juice, pH levels vary depending on the type of fruit, its ripeness, and the processing methods used [25]. The pHs of fruit juices may also affect their biological activities, including their antimicrobial properties. Ascorbic acid plays a crucial role in various physiological functions in the human body, including collagen formation, wound-healing, and boosting the immune system. In this study, amla has been shown to be a good source of ascorbic acid, with concentrations of 540 mg/100 g. Carbohydrates are the most abundant macronutrient in fruit juices and are important sources of energy for the body. In this

Table 9. MIC value for antibacterial fruit juices against UTIs & STIs pathogen

Microbial isolates	MIC 50 of fruits juices (%)			
	Lemon	Amla	Pineapple	Mosambi
E. coli	20	20	40	100
K. pneumoniae	20	40	60	100
P. mirabilis	20	40	60	100
P. aeruginosa	20	20	60	100
N. gonorrhoea	20	40	60	100
Ureaplasma spp.	20	40	40	100

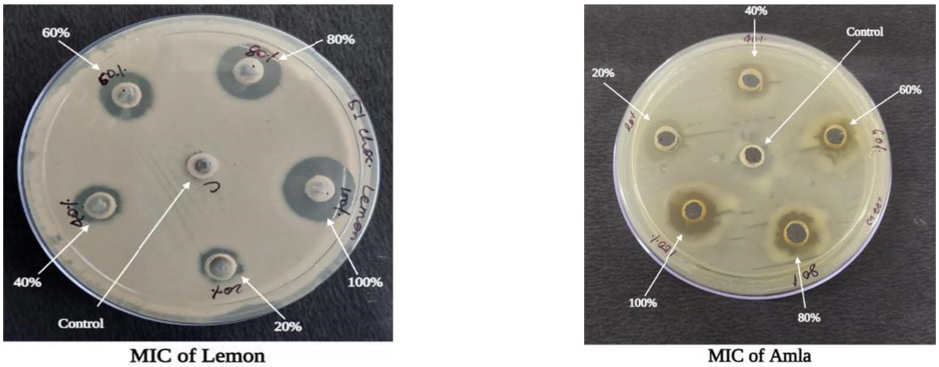

Figure 2. Minimum Inhibitory Concentration (MIC) of lemon and amla juices against *N. gonorrhoea*.

study, total carbohydrate contents ranged from 15.28 g/100 g in pomegranate juice to 4.80 g/100 g in mosambi juice.

In 2019, Tewari et al. [22] found that amla fruit juice contained 0.39% fat, 87.04% moisture, and 77.18% carbohydrate. The results of this study showed that the fat (0.30 g, equivalent to 30%) and moisture (84%) contents in amla were similar those reported by Tewari et al. [22], but the carbohydrate content was much lower (6.94 g/100 g, equivalent to 6.94%). A study by Hussain et al. [26] reported a carbohydrate content of 14 g/100 g for amla, while Gul et al. [27] reported carbohydrates of 7.6-70 g/100 g dry fruit weight; however, the amount of carbohydrate in amla fruit has been shown to depend on its ripening state and fruit age [28]. Liu et al. [29] reported fat (0.30 g/100 g), protein (1.10 g/100 g), carbohydrate (9.32 g/100 g), and fiber (2.80 g/100 g) contents in lemon [29]. The results of this study closely corresponded to those of Liu et al. [28] with the following content measurements: fat (0.32 g/100 g), protein (0.98 g/100 g), carbohydrate (7.25 g/100 g), and fiber (8.81 g/100 g). This study showed that oranges contain 8.13 g/100 g of carbohydrates, 0.83 g/100 g of protein, 0.17 g/100 g of fat, and 2.78 g/100 g of crude fiber. Similar findings were reported by Hussain et al. [26], who observed a carbohydrate content of 12.54 g/100 g, 0.94 g/100 g of protein, 0.12 g/100 g of fat, and 2.40 g/100 g of fiber in oranges. They also analyzed the chemical components of pomegranate and reported the following contents: carbohydrate, 18.7 g/100 g; protein, 1.67 g/100 g; fat, 1.17 g/100 g; and crude fiber, 4 g/100 g. The present study revealed that pomegranates contain 15.28 g/100 g of carbohydrates, 1.65 g/100 g of protein, 1.28 g/100 g of fat, and 3.80 g/100 g of crude fiber.

For kiwi, this study showed a carbohydrate content of 11.65 g/100 g, protein content of 1.21 g/100 g, fat content of 0.67 g/100 g, and crude fiber content of 3.42 g/100 g. These findings are similar to those reported by [30], who found that kiwi has a carbohydrate content of 14.66 g/100 g, protein content of 1.14 g/100 g, fat content of 0.52 g/100 g, and crude fiber content of 3 g/100 g. Hossain et al. [31] reported that pineapple contained 87.3% moisture, 13.7 g of carbohydrates, 0.54 g of protein, and 1.40 g of dietary fiber. This study found 86% moisture, 11.72 g of carbohydrates, 0.58 g of protein, and 1.23 g of dietary fiber in pineapple. Another report determined that mosambi contained 5.20 g of carbohydrates, 0.21 g of fat, protein of 0.77 g, and fiber of 2.07 g [32]. In the present study, it was determined that it contained approximately 4.80 g of carbohydrates, 0.21 g of fat, 0.77 g of protein, and 2.70 g of crude fiber per 100 g of fresh fruit.

Lemon juice exhibited the highest antibacterial activity against all clinical isolates of the UTI and STI pathogens, followed by amla and pineapple. Although the antibacterial activity of lemon juice has been reported previously [33-35], this is possibly the first report of its use against MDR bacterial isolates involved in UTIs and STIs. In this study, lemon juice exhibited the highest antibacterial activity against all six MDR pathogens tested; namely, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *N. gonorrhoea*, and *Ureaplasma* spp.

The antibacterial activity of lemon juice may be due to its low pH largely caused by its high citric acid content; the acidic environment around the well on the agar plate likely inhibited bacterial growth. Pomegranate juice, with a relatively high pH, was inactive against the studied bacteria. Chemical analyses of the juices also indicated that pomegranate juice had the highest carbohydrate content, which may also explain its lowest antibacterial activity. Its high carbohydrate content and other micronutrients of pomegranate may have provided additional nutrients for the bacteria. Amla fruit juice also exhibited good antibacterial activity against the selected MDR strains of pathogenic bacteria in this study. Amla has a number of pharmacological activities, such as anticancer, analgesic, antipyretic, antioxidant, cardiotonic, cerebral tonic, intestinal tonic, and antimicrobial activities [33, 34]. The low pH of its juice and the presence of tannins may be responsible its strong antibacterial activity. Apart from UTI pathogens, lemon, amla, and pineapple juice were active against *N. gonorrhoea* and *Ureaplasma* spp. Management of STIs using present-day antibiotics is difficult. The activities of these fruit juices against STI-causing bacteria provides many options for treatment of drug-resistant bacterial infections.

MIC_{50} is a measure of the susceptibility of a microorganism to an antimicrobial agent and represents the concentration at which 50% of the organisms in a test population are inhibited [36].

The MIC_{50} of lemon juice was at a concentration of 20% for all organisms tested. The MIC_{50} of amla juice occurred at a concentration of 20% for *E. coli* and *P. aeruginosa* and at 40% for other selected bacteria. The MIC_{50} of pineapple juice was at a concentration of 40% for *E. coli* and *Ureaplasma* spp. and 60% for the other selected bacteria. Diluted mosambi juice did not show any activity; hence, the MIC_{50} was estimated to be at a concentration of 100%.

Cell adhesion is necessary for effective colonization by bacteria and a critical step in the pathophysiology of infection.

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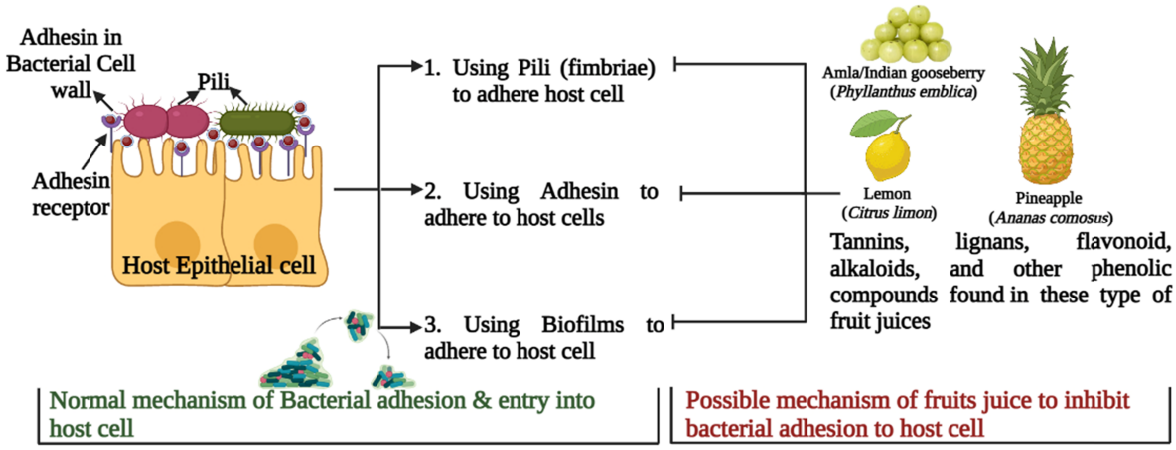

Figure 3. Possible mechanism of antibacterial activity of lemon, amla and pineapple fruit juices.

Adhesion entails contact between the surfaces of the host and bacterial cells [37]. Tannins, lignans, flavonoids, alkaloids, and other phenolic compounds present in lemon, amla, and pineapple fruit juices (Fig. 3) may be responsible for loss of bacterial cell adhesion, reducing their pathogenicity. Moreover, these compounds may alter cell surface structures and integrity in a way that masks adhesin molecules present on the bacteria or on the receptors present on host epithelial cells [38].

CONCLUSION

Lemon and amla juices showed the highest activities against MDR UTI and STI pathogens. The low pHs of lemon and amla juices may be responsible for their strong antibacterial activities. The findings of this study suggest that lemon and amla juices may be cost-effective, safe, and easily available sources of antibacterial agents for UTI and STI management. Isolation and purification of bioactive constituents from these juices may provide valuable drug leads for effective management of MDR bacterial pathogens involved in UTIs and STIs.

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AUTHORS' CONTRIBUTIONS

Prof. Poonam Sharma conceptualized, designed, supervised the study and corrected the manuscript. Ms Juhi performed experiments related to fruit juice extraction, physiochemical estimations and drafted the manuscript. Ms Vaishali Halwai and Ms Sainivedita Rout performed antibacterial activity experiments. Prof. Rambir Singh analysed and interpreted the results and edited the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

ETHICAL APPROVAL

The work is institutional ethical committee Indira Gandhi National Tribal University, Amarkantak, under Ref. No., IGNTU/IEC/01/2019, dated 11/05/2019 and Govt. Medical College Shahdol under Ref. no. IERC/22/06/001, dated 16/06/2022.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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