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Antimicrobial Properties of Syzygium Aromaticum Extract Against Common Uropathogenic Bacteria

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Abstract: Urinary tract infections (UTIs) cause a large burden of disease affecting 150-250 million people per year worldwide the study involved 400 urine samples from patients suspected of having UTIs who visited tertiary-care hospitals, private hospitals, and pathology centers in Rewa District from October 2021 to September 2023. Antibiotic resistance is a pressing issue, necessitating new treatments against MDR microorganisms. Essential oils, being multi-component, may offer new options to combat microbial resistance, potentially outperforming conventional antimicrobials that target a single site, particularly in E. coli.

Keywords: Antimicrobial, Syzygium, Aromaticum, Bacteria, infection

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INTRODUCTION

Throughout a person's lifespan, they are exposed to several infectious illnesses. One such condition is a urinary tract infection (UTI), which affects about 10% of the population and, if left untreated, may cause serious health complications. Uropathogens comprise a wide variety of microbes, including viruses, fungi, and bacteria; nevertheless, bacteria are the most common causative agent in almost all instances of UTI (95%). When bacteria infect the mucosa lining the urinary bladder, ureters, and/or renal pelvis, a urinary tract infection (UTI) develops. Anatomical anomalies, incontinence, sexual and anal intercourse, immunosuppression, menopause, and many more conditions are among the numerous that might increase the risk. Sepsis, kidney damage, and issues like Clostridium difficile colitis and high-level antibiotic resistance are some of the serious consequences that may arise from the overuse of antibiotics. The high recurrence rate and the prevalence of multidrug-resistant (MDR) strains have made UTI a significant economic and social burden.

The current gold standard for diagnosis relies on a combination of clinical symptoms, murky urine, and a positive dipstick test; in some cases, further testing using cultures and microscopes may be necessary. (Smelov et al., 2016). According to the RCPA (2021), The presence of 103 or more bacterial colony forming units per millilitre (CFU/ml) in the midstream of the urine, in addition to other clinical signs, is indicative of a urinary tract infection (UTI). Nevertheless, it is important to note that not all types of UTIs and conditions may be definitively diagnosed with a fixed bacterial count for substantial bacteriuria **(Hooton TM, *et al.*,2013).** Serious differences have been observed in UTI testing, therefore many

infections including lower bacterial counts, intracellular bacterial communities (IBCs) and/or biofilms may be overlooked**.**

LITERATURE REVIEW

(WolfeA J & Brubaker L, 2015). Urine from healthy people either doesn't contain any microbes at all or has a very low quantity of the harmful kind. Some examples of UTIs include cystitis (in the bladder), urethritis (in the urethra), and pyelonephritis (in the kidneys). Among the most frequent infectious illnesses globally, they impact 150 million people yearly, causing a high incidence of morbidity and expensive medical bills (for instance, repeated UTIs in the US are projected to cost over $5 billion annually). Although the specific symptoms experienced by individuals with UTIs differ according on the site of infection, it is well-documented that these illnesses significantly diminish patients' quality of life by negatively affecting their personal and professional relationships.

(McCann E, et al, 2020) A urinary tract infection (UTI) may be either simple (uUTI) or complex (cUTI). Urinary tract infections (uUTIs) usually strike healthy individuals who do not have any neurological or anatomical issues with the urinary system. Complex cUTIs includes other urinary tract abnormalities that make the patient more likely to get an infection. These abnormalities can be either functional or anatomical, and they include things like catheterisation or obstructive uropathy, neurogenic bladder, renal failure, pregnancy, or calculi.

(Huang L, *et al.*, 2021) Common types of UTIs include urethritis, the urethra, the ureter, the kidneys, and the bladder, respectively; pyelonephritis and cystitis occur in this group. The presence of infection-prone conditions or the nature of the event (primary or recurring) are two other factors that determine the classification of UTIs.

(VanBuulL W, et al., 2018) Low oestrogen levels (i.e., fewer helpful lactobacilli), diabetes, incontinence, protrusion of the vaginal wall, and inadequate bladder emptying are the primary risk factors linked with UTI recurrence. Infection in the upper urinary system, or pyelonephritis, often manifests with a high temperature, chills, nausea, discomfort at the costovertebral angle, and vomiting. Because of the vast differences in treatment and prognosis between simple and complex pyelonephritis, it is critical to differentiate between the two.

According to Gomila A. et al. (2018), a novel family of medications comprises plazomycin, cefiderocol, meropenem-vaborbactam, and imipenem/cilastatin. Complex infections that are resistant to carbapenems may have a new therapy option in these novel medicines. The clinical treatment of cUTI is based on the severity of the illness upon presentation.An intravenous antibiotic regimen including amoxicillin and an aminoglycoside, a second-generation cephalosporin and an aminoglycoside, or a third-generation cephalosporin with or without an aminoglycoside should be administered to patients as a first line of defence. Combinations such as Ceftolozane/tazobactam, Ceftazidime/avibactam, and Imipenem/cilastatin provide an alternative to the usual treatment for cUTIs caused by MDR bacteria.

RESEARCH METHODOLOGY

The present study "A Study on Bacterial Uropathogens with reference to Antimicrobial activity of

Syzygium *aromaticum*" was conducted in the Microbiology laboratory of Centre for Biotechnology and Microbiology studies, A.P.S. The present study included 400 urine samples collected from patients, who visited tertiary-care hospital (SGMH), private hospitals and pathology centers in Rewa District between the period from October 2021 to September 2023 suspected of having UTIs. In each 30 ml Fresh midstream urine specimens were collected in sterile plastic bottle from different patients of various age groups and sex attending SGMH, private hospitals and pathological laboratories. A triple-axis detector on a 5975C VL MSD mass spectrometer from the same brand as the Agilent Technologies 6850 Network GC System chromatograph and an RTX capillary column made of dimethyl-polysiloxane (95%) and phenyl polysiloxane (5%) measuring 30 meters in length and 0.25 mm in internal diameter were used.

DATA ANALYSIS

Sample collection:

During the research period from October 2021 to September 2023, a total of 3,650 patients with UTI complaints were seen at the tertiary-care hospital in Rewa City (M.P.). Their urine samples were collected for culture and sensitivity testing. Of them, 210 samples (5.6%) had fungal or mixed growth, 3040 samples (83.3%) showed no growth, and 400 samples (10.9%) produced substantial bacteriuria. (Referring to Table 1) The A.P.S. University research committee gave its stamp of approval, stating that the study followed all applicable ethical standards.

Table 1: Prevalence of Culture Positive cases (bacterial infection) among patients.

Total number of urine samples	No of samples with significant bacteriuria	No of samples without significant bacterial growth	No of samples with fungal/mixed growth
3650	400(10.9%)	3040(83.3%)	210(5.8%)

Table 2: Distribution of Culture Positive cases by Age and Sex

Distribution of Cases by Age	Distribution of Cases by Sex		Total	Percent
	Male	Female		
0-14 years	20(27%)	58(18%)	078	19.5%
15-40 years	18(24%)	27(08%)	045	11.25%
41-60 years	15(20%)	90(28%)	105	26.25%
≥61	22(29%)	150(46%)	172	43%
	75 (18.75%)	325 (81.25%)	400	

Table 2, is showing that out of 400 culture positive urine samples, 325(81.25%) cases were collected from females. In addition, the maximum number of patients belonged to the age group 61years and above (172 cases 43%) and least cases belonged to the age group 15-40 years(11.25%).

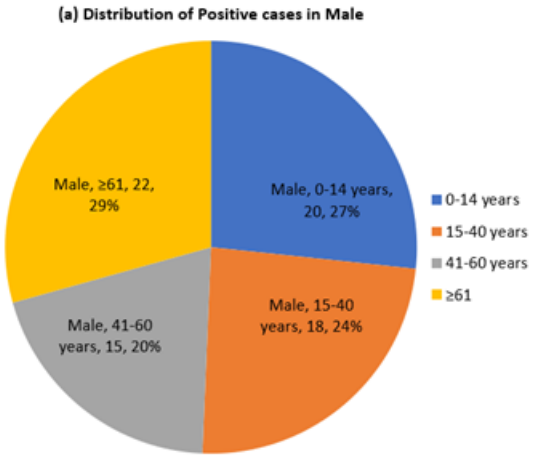

Figure is showing that out of 400 culture positive urine samples, 75(18.75%) cases were collected from males. Maximum number of patients belonged to the age group 61years and above (22 cases 29%) and least number of cases belonged to the age group 41-60 years (20%).

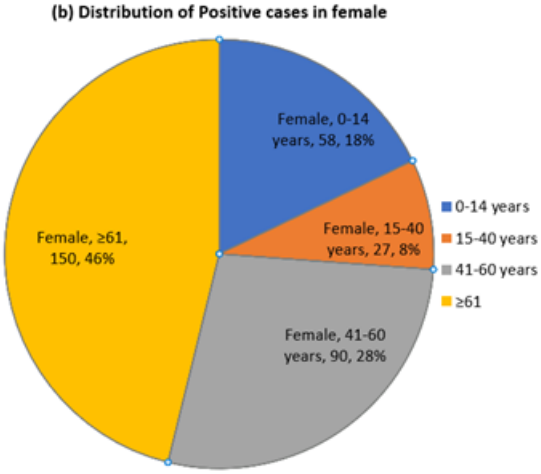

Figure is showing that out of 400 culture positive urine samples, 325(81.25%) cases were collected from females. In addition, the maximum number of patients belonged to the age group 61years and above (150 cases 46%) and least cases belonged to the age group 15-40 years (08%).

Table 3: Biochemical Tests Results

S. No.	Catalase	Oxidase	TSI Test				Indole	MR	VP	Citrate	Urease	Motility	Nitrate	PPA	Assumed microorganisms
			But	Slant	Gas	H2S									
1	+	-	Y	Y	+	-	+	+	-	-	-	+	+	-	*E.coli*
2	+	-	Y	Y	+	-	-	-	+	+		+		-	*K.pneum*
3	+	-	Y	Y	-	-	-	-	+	+	-	+	+	-	*Entrobacter aerogenes*
4	-	-	Y	Y	+	-	-	-	+	-	-	-		-	*Proteus*
5	+	+	K	NC	-	-	-	-	-	+	-	+	+	-	*Pseudomonas* spp.

Table 4: Distribution of Uropathogens

Uropathogens		Total (400)
Escherichia coli isolates		240(60%)
Non-E. *coli* isolates	*Klebsiella pneumonia*	80(20%)
	Pseudomonas aeruginosa	50(12.5%)
	Enterobacter species	20(5%)
	Proteus species	10(2.5%)

Out of 400 culture positive patient urine samples from those with a clinical suspicion of having UTIs, The *E. coli* was the chief uropathogen and accounted for 60% (240/400) of the positive culture in both the genders. (V. Niranjan and A.Malini, 2014)

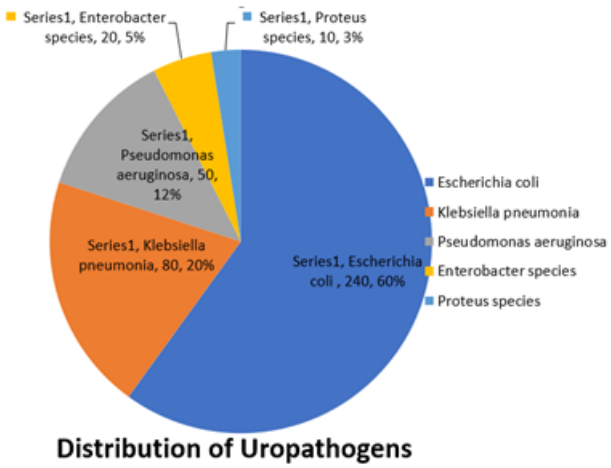

Table 5: Disc Diffusion Method for Antibiotic Resistance Patterns in Bacterial Isolates

Antibiotics	Std. Range			*E. coli*	*K. pneumo*	*P. aerug.*	*Entero. aerogens*	*Proteus species*	Total	Statistical significance
				N=240	*N=80*	*N=50*	*N=20*	*N=10*	*N=400*	
	Sensi	Int	Resis							
Ampicilin(10 µg)	17	14 - 16	13	230 (95.8 %)	72 (90%)	47 (94%)	19 (95%)	10 (100%)	378 (94.5 %)	P<0.0001
Cefoxitin(30 µg)II	18	15 - 17	14	117 (48.8 %)	34 (42.5 %)	30 (60%)	10 (50%)	03 (30%)	194 (48.5 %)	
Ceftazidime (30 µg)III	21	18 - 20	17	180 (75%)	68 (85%)	43 (86%)	19 (95%)	08 (80%)	318 (79.5 %)	
Ceftriaxone(30 µg)III	23	20 - 22	19	167 (69.6 %)	49 (61.3 %)	42 (84%)	15 (75%)	09 (90%)	282 (70.5 %)	
Cefepime(30 µg)IV	25	19 - 24	18	153 (63.8 %)	51 (63.8 %)	45 (90%)	18 (90%)	09 (90%)	276 (69%)	
Norfloxacin(10 µg)	17	13 - 16	12	110 (45.8 %)	47 (58.8 %)	50 (100 %)	13 (65%)	07 (70%)	227 (56.8 %)	
Gentamicin(10 µg)	15	13 - 14	12	208 (86.7 %)	76 (95%)	50 (100 %)	20 (100%)	08 (80%)	354 (88.5 %)	
Meropenem (10 µg)	23	20 - 22	19	02 (0.83 %)	00 (00%)	00 (00%)	00 (00%)	00 (00%)	02 (0.5%)	

Table 5 shows the antimicrobial resistance pattern of all isolates which were isolated from urine samples. Ampicilin had a 94.5% success rate against E. coli and other uropathogens, whereas Gentamicin had an 88.5% success rate. All of the isolates showed resistance to third-generation cephalosporins, such as Ceftazidime and Ceftriaxone, ranging from 79.5% to 70.5%. Alternatively, Meropenem resistance was found in just 2% of the isolates.

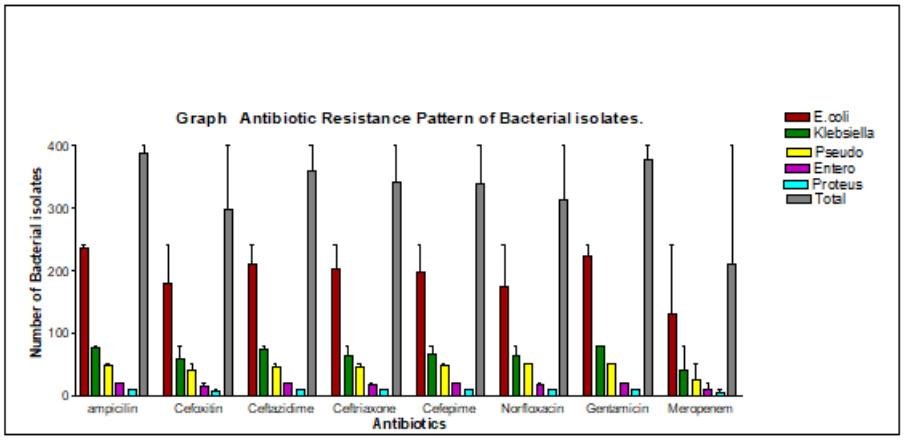

Table 6: Phenotypic Screening and Distribution of isolates

S.N.	Type of Bacterial species	Number of isolates	Isolates showing Resistance				Non Resistant isolates	
			ESBL-producing strains	AmpC-producing strains	Total	%	Non ESBL/ AmpC producing strains	%
1	*Escherichia coli*	240	50(12.5)	20(5)	70	17.5%	170	42.5%
2	*Klebsiella pneumonia*	80	15(3.75)	7(1.75)	22	5.5%	58	14.5%
3	*Pseudomonas aeruginosa*	50	10(2.5)	00(00)	10	2.5%	40	10%
4	*Enterobacter aerogens*	20	04(1)	0(00)	04	1%	16	4%
5	*Proteus species*	10	03(0.75)	00(00)	03	0.75%	07	1.75%
	Total	400	82	27	109	27.25 %	291	72.75 %

From the table 6, it is clear that on Phenotypic Screening, most prevalent number of isolates was *E. coli* showing ESBL and AmpC resistance patterns were found with rates of 12.5% and 5%, correspondingly, which ranked first among other isolated resistant bacterial strains.

CONCLUSION

Ever since its debut, antibiotic therapy—one of the most crucial tools in the battle against infectious diseases—has greatly improved people's health There has been progress in this treatment, but we are still at a time when antibiotic-resistant illnesses are worryingly on the increase. The emergence of different types of resistance in bacteria in response to the introduction of new antibiotics for therapeutic use casts doubt on the function of antibiotics in nature Worldwide, and especially in underdeveloped nations, infections caused by beta-lactamase-producing bacteria remain a major public health concern. New treatments against MDR microorganisms are urgently needed due to the prevalence of antibiotic resistance. As a result, the antibiotic's side effects are lessened. Most significantly, there may be new options to combat the invasion of microbial resistance if essential oils are used with medicines to target resistant bacteria. There could be a variety of ways in which these oils work. Due to the fact that essential oils are complex mixtures, they are thought to be more effective than many conventional antimicrobials that only target a single site in avoiding bacterial resistance in E. coli, the bacteria that cause UTIs.

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