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Antibacterial activity of *Azima tetracantha L.* against isolated aminoglycoside resistant bacteria

Thaha Thariq J*, Prince L
Department of Microbiology, Marudupandiyar College (Affiliated to Bharathidasan University), Thirucirappalli, Thanjavur, Tamilnadu, India

Abstract
Urinary tract infection (UTI) is the most common hospital acquired infection, accounting for 40% of all hospital acquired infections. In this present study were carried out antibacterial activity of medicinal plants against isolated aminoglycoside resistant bacteria. The UTI urine samples were collected from various hospitals and collected samples were inoculated on HiCrome Differential Agar for isolation of UTI pathogens. Isolated aminoglycoside resistant bacteria were identified based on cultural and morphological characteristic. The antibiotic sensitivity of isolated aminoglycoside resistant bacteria to the commercial antibiotic tests was analyzed by disc diffusion method. *Azima tetracantha L.* phytochemical compounds were carried out on the aqueous, acetone and methanol extract the powdered specimens using standard procedures. GC-MS analysis also studied in *Azima tetracantha L.* plant extract. Among this study different phytochemical compound were identified, as per the reports of earlier literature, these phytochemical compounds are known to have various medicinal properties.

Keywords: urinary tract infection, aminoglycoside, *Azima tetracantha L.*

Introduction

Urinary tract infections (UTI) known as the propagation of active microorganisms within the urinary channel and its causes to the environment. The Bacteria are presence in the urine is called bacteriuria. UTI's usually separated into two major types, such as symptoms and asymptomatic bacteriuria. The *Escherichia coli* from the gut were infected to 80 to 85% of community acquire UTIS and *Staphylococcus saprophyticus* was caused to 5 to 10 %. Infrequently do they may be also cause to viral or fungal infections. Healthcare-associated urinary tract infections involve a much broader range of infective agents such as *Escherichia coli* (27%), *Klebsiella pneumonia* (11%), *Pseudomonas aeruginosa* (11%), *Enterococcus* sp. (7%) and fungal agent *Candida albicans* (9%). Innovation of antibiotics was one of the greatest advances of current medicine but the ease of use and increased use of antibiotics slowly lead to microbial resistance to them. Antimicrobial resistance is increasing around the world, especially in developing countries like India [1].

According to the World Health Organization in 2014, antimicrobial resistance is increasingly a global threat for public health and all countries have focused on this problem which is a serious threat to modern medicine. Regarded as nosocomial pathogen of the 1990s, enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. Serious UTI infections are often refractory to treatment and the mortality is high [2]. Infections by enterococci have traditionally been treated with cell wall active agents (e.g., penicillin or ampicillin) in combination with an aminoglycoside (streptomycin / gentamicin), however emergence of high level resistance to aminoglycosides (HLAR), β lactam antibiotics and to vancomycin by some strains, together with association of HLAR with multi drug resistance has led to failure of synergistic effects of combination therapy [3]. Streptomycin was the aminoglycoside used clinically until 1970 when more than 50% of enterococci were found to be resistant to high level of this drug. High level gentamicin resistance (HLGR) was first reported in *E. faecalis* 1979 [4].

Azima tetracantha L is the most important Indian medicinal plant used in the treatment of various diseases. Although some chemical constituents of its plant have been isolated earlier and their chemical structures elucidated, detailed investigation to identify the chemical constituents responsible for the curative action of these crude drugs has not yet been reported. Numerous tropical medicinal plants are used traditionally which are remedial against these diseases [5]. Medicinal and aromatic plants and their essence are rich in antibacterial and antifungal compounds could be an alternate way to combat against bacterial and fungal diseases [6]. Therefore, the present investigation was undertaken antibacterial activity of *Azima tetracantha L.* against isolated aminoglycoside resistant bacteria.

Materials and Methods

To isolate UTI aminoglycoside resistant bacterial strains, sample of urine was collected from patients hospitalized in the various hospitals in Thanjavur during a 12 month period (September 2018 – August 2019). Pure strains obtained from the growth of each sample on HiCrome™ UTI Agar (HiMedia M1418), Blood agar (HiMedia M073) and MacConkey Agar (HiMedia MH081) plates at 37°C were examined using conventional methods to identify the genus and species. To determine the genus, Gram staining, motility, catalase test, growth in the presence of 6.5% NaCl, bile esculin test and PYR (L-pyrolidonyl naphthylamidase) were evaluated. In addition, the species type of each isolate was identified using biochemical tests and sugar fermentation patterns of arabinose, sorbitol, mannitol, sorbose and sucrose were assessed [7].

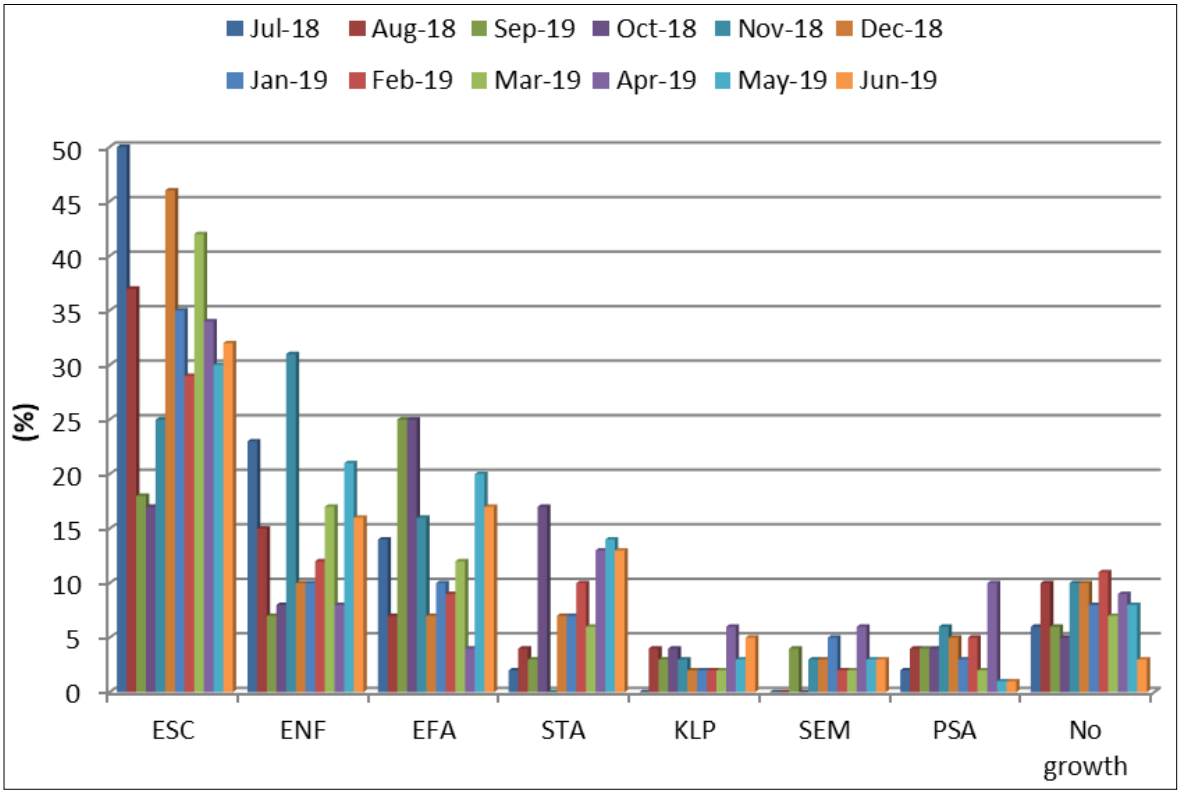
The antimicrobial susceptibility patterns to seven antibiotics, including ampicillin, gentamicin, vancomycin, chloramphenicol, ciprofloxacin, erythromycin and tetracycline, were investigated for all isolates using the disc diffusion method (Kirby-Bauer) according to CSLI guidelines (Bootle, Mast Mersey Side, UK). In addition, the micro-dilution method was used to screen for HLGR strains (MIC≥500g/ml). Interpretation of the obtained results and the MIC determination was performedaccording to CSLI guidelines (8-1024g/ml) [8]. The present study was carried out in the leaves of the plant, *Azima tetracantha L*. The plant was authenticated and certified by Department of Botany, St. Joseph's College (Autonomous). Tiruchirappalli, India and also by Dr. S. Soosairaj, Botanist, Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli. The specimen number is 2021/3000. The powdered leaves plant sample (20g) was soaked and dissolved in 75 ml of methanol for 24 h separately. Then the filtrates were collected and evaporated under liquid nitrogen [9]. The prepared plant materials were analysis the phytochemical compounds from different extracts by carried out qualitatively standard method [10, 11] and analysis the *Aristolochia indica L* medicinal plant active compounds by GC-MS. Assay the antibacterial activity against isolated UTI aminoglycoside resistant bacteria was carried out using disc diffusion method [12].

Results and Discussion

Aminoglycosides are broad-spectrum antibiotics of high potency that have been traditionally used for the treatment of serious Gram-negative infections. Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents [4].

HiCrome UTI agar was chromogenic medium that make easy rapid isolation in addition to presumptive identification of most UTI bacteria including various species from urine. In the present investigation 536 urine sample collected from July 2018 to June 2019, UTI patients out of 443 (83%) were showed urine culture positive 93 (17%) patients urine culture negative (Figure 1). On the topic of UTI and polymicrobial growths from urine culture, our results agree with a few studies were done in India [13]. The present investigation maximum level of positive urine infected bacterium is *Escherichia coli* (35%). Ciragil *et al.* [14] have been noted 20 to 30% of UTI urine samples results in significant growth with main infective bacteria like *Escherichia coli* in both community and hospital acquired infections [15].

Urinary tract infections caused by *Klebsiella pneumoniae* appear to be rising and have been become a real health problem, especially in hospital settings [16].



ESC - *Escherichia coli;* ENF - *Enterococcus faecium;* EFA - *Enterococcus faecalis;* STA- *Staphylococcus aureus;* KLP - *Klebsiella pneumoniae;* SEM- *Serratia marcescens;* PSA- *Pseudomonas aeroginosa*

Fig 1: Isolation of UTI bacteria

The *Enterococcus faecium* (16%) was positive urine infected bacterium, a similar laboratory-based study conducted in France found that *Enterococcus faecium* accounted for only 13% of isolates from UTI specimens [17]. In this study, *Serratia marcescens* (3%) was found to be the isolate in UTIs. The most of the investigation were done on *Serratia* support the similar results which could be attributed to the dominance of *Serratia marcescens* in the body flora of the humen [18]. This finding was similar with the study of Tayebi *et al.* [19], Jombo *et al.* [20] in which *Serratia sp*. was 8.7% and 12.4%, respectively. The most common organism isolated in these patients was *Escherichia coli* (35%), *Enterococcus faecium* (20%), *Enterococcus faecium* (12%), *Staphylococcus aureus* (9%),

Klebsiella pneumoniae, (3%), *Serratia marcescens* (3%) and *Pseudomonas aeroginosa* (4%) (Table 1). This findings pattern was similar with study of Savitha and Thangamariappan like *Escherichia coli* (48.04%), *Klebsella* (8.82%), *P. aeruginosa* (0.98%), *Proteus. spp* (4.9%) and Gram positive organisms (37.26%) [21].

Table 1: Prevalence of the isolated bacteria in collected urine samples

Bacteria	Observation	
	No. of Positive UTI	Percentage (%)
Escherichia coli	187	35
Enterococcus faecium	83	16
Enterococcus faecalis	74	13
Staphylococcus aureus	48	9
Klebsiella pneumoniae	16	3
Serratia marcescens	15	3
Pseudomonas aeroginosa	20	4
No Growth	93	17
Total Number of Samples	536	

The worldwide problem of antibiotic resistance was rapid and becoming one of the most important scientific problem of current era. Phenotypic resistance to aminoglycoside antibiotics as analyzed by standard Kirby-Bauer disc diffusion method showed in table 2. Aminoglycoside resistance rates, phenotypes, and mechanisms of Gram-negative bacteria from infected patients across the world have shown progressive trends and it has been observed that resistance patterns have been disseminated in various bacterial species [22]. We screened resistance to aminoglycoside antibiotics using phenotypic method. These results clearly indicate the enhanced resistance in the bacterial isolates against various aminoglycoside antibiotics. An earlier report that has employed Kirby-Bauer method to check the high level aminoglycoside resistance in *Enterococci* isolates has provided the similar inference for aminoglycoside resistance against gentamicin, streptomycin, and both of them together from Chennai, India [23]. Resistance to aminoglycosides in enterococci is often associated with multidrug resistance. In our study, *E. faecalis* and *E. faecium* showed resistance to as many as five drugs (Table 2).

Table 2: Distribution of the high level aminoglycoside resistance bacterial species with respect to resistance to aminoglycoside Antibiotic

Bacteria	Zone of Inhibition (mm in diameter)				
	Amikacin	Gentamicin	Kanamycin	Streptomycin	Tobramycin
Escherichia coli	10±1.43	-	-	-	-
Enterococcus faecium	11±1.40	-	-	12±1.76	-
Enterococcus faecalis	08±1.41	-	11±1.07	08±1.40	14±1.21
Staphylococcus aureus	10±1.21	-	10±1.47	-	-
Klebsiella pneumoniae	-	-	-	-	-
Serratia marcescens	-	-	-	-	-
Pseudomonas aeroginosa	-		-	-	-

Recently, much concentration has been focussed towards plant extracts and biologically active compounds separated from medicinal plant species. Hamburger and Hostettmann [24] have been investigated that the whole number of plant chemicals might go above four lacks and out of it more than ten thousand were secondary metabolites whose most important function in plant was suspicious in nature. Several researches have been reported that many plants possess antimicrobial properties including the parts which include such as leaf, stem, bark and flower. It has been shown that when solvents like ethanol, acetone and methanol are used to extract plants, most of them are able to exhibit inhibitory effect on bacteria [25]. The flavonoids and saponins were present in all the extracts of *Azima tetracantha L* plant leaves. The flavonoids have been reported to have antimicrobial properties [26]. The phyto flavonoids and phenolic compounds are responsible for potential for antioxidant activity and also effective for the prevention of various diseases [27].

The tannins phytochemical have antimicrobial activity [28] and antioxidant properties. Tannins and terpenoids were presented in leaves acetone and methanol extract. Phlobatannis, steroids and glycosides was present in methanol extracts only (Table 3).

Present investigation also corroborates the occurrence of saponin by qualitative test, as they are usually considered as the soapy substances that are general cleansers, having antiseptic properties [29].

Table 3: Phytochemical screening of *Azima tetracantha L*

Phytochemical Compounds	Observation			
	Control	Aqueous	Acetone	Methanol
Tannins	-	-	+	+
Flavonaids	-	+	+	+
Terpenoids	-	-	+	+
Saponins	-	+	+	+
Phlobatannins	-	-	-	+
Steroids	-	-	-	+
Carbohydrates	-	-	-	-
Glycosides	-	-	-	+
Cournarins	-	-	-	-
Proteins	-	-	-	-
Emodins	-	-	-	-
Anthraquinones	-	-	-	-
Anthocyanins	-	-	-	-
Leucoantho cyaninsturns	-	-	-	-

Forty compounds were identified in the methanol extract of *Azima tetracantha L* leaves. Chromatogram with the peaks of the test compounds with respect to retention time is shown in Figure 2. The prevailing compounds as per the peak report were 2-Methyl-2-Nonene, Dodecane, Neophytadiene, 2-Pentadecanon, 6,10,14-Trimethyl-, 5,9,13-Pentadecatrien-2-One, 6,10,14-Trimethyl-, (E,E)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, Sulfurous acid, cyclohexylmethyl hexyl ester, Phytol, γ-Sitosterol, β-Sitosterol, Friedelan-3-one, and α-Tocospiro. Earlier reports revealed methoxylated flavones, apigenin 7-methyl ether and apigenin 7,4'-dimethyl ether from *Azima tetracantha L* and chrysoeriol 7-O-glucuronide and acacetin only in *Azima tetracantha L* [30] and additionally, a dimethyl ether flavonol was found in *Azima tetracantha L* kaempferol, 40-dimethyl ether [31].

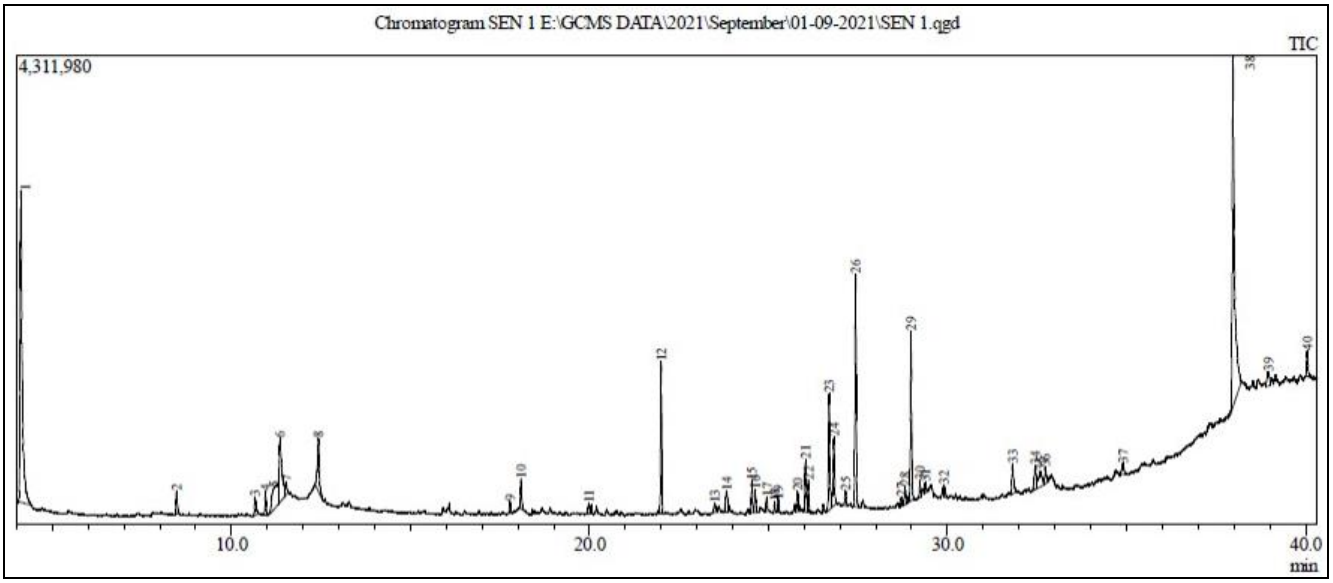


Fig 2: *Azima tetracantha L.* plant leaves methanolic extract phytocompounds were confirmed by GC-MS.

Antibacterial activity of *Azima tetracantha L* was analyzed against isolated UTI aminoglycoside resistant bacteria. The maximum antibacterial activities were observed in methanol extract other than aqueous extract and ethanol extract. Among the three bacterial organisms maximum growth suppression was observed in *Escherichia coli* (24±1.35 mm in diameter) and *Staphylococcus aureus* (24±1.57 mm in diameter) when compared with other isolated UTI aminoglycoside resistant bacteria (Figure 3). Hema *et al.* [32] have shown that *Azima tetracantha L* leaf inhibit the growth of the clinical pathogens. Previously Anbukumaran *et al.* [33] reported *Azima tetracantha* showed highest antibacterial activity on different concentration of ethanol when compared to methanol and water extracts. Specific plant compounds such as anthraquinones [34] and dihhydroxyanthraquinones as well as saponins [35] have been proposed to have direct antimicrobial activity. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, antiallergenic, antispasmodic, antihyperglycemic, antiinflammatory and immunomodulatory properties [36, 37]. Glycosides also have vast therapeutic efficacy as they are found in almost methanol extract.

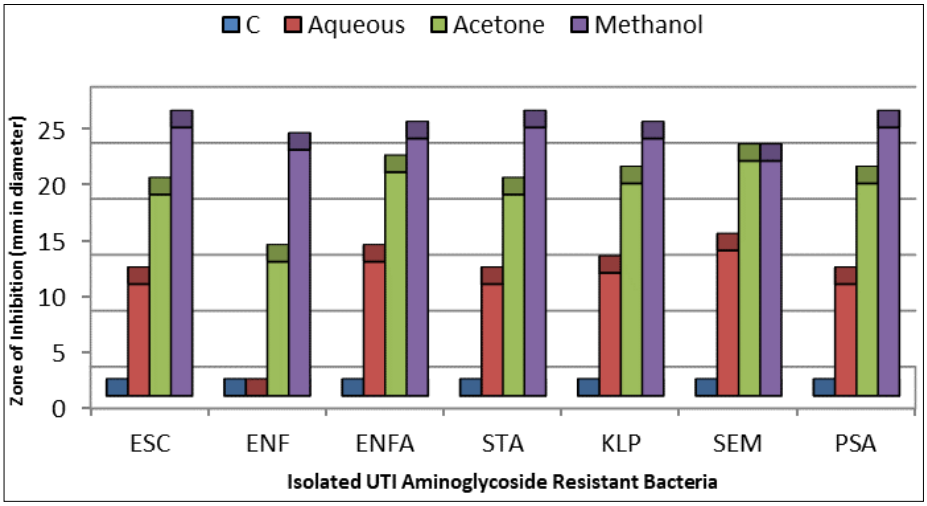


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Fig 3: Antibacterial bacterial activity of *Azima tetracantha L* against isolated UTI aminoglycoside resistant bacteria

Conclusion

In present investigation revealed that antibacterial activity of *Azima tetracantha L.* against isolated aminoglycoside resistant bacteria. We screened resistance to aminoglycoside antibiotics from isolated UTI bacterial strains. The majority common bacterial strains *Escherichi*a *coli*, *Enterococcus faecium*, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Serratia marcescens* and *Pseudomonas aeroginosa* were identified. In present investigation revealed that justification for the use of the plant *Azima tetracantha L.* as a folk medicine. Antimicrobial potential and GC-MS analysis of the plant *Azima tetracantha L.* leaves is the first of its kind. In present study, methanolic crude extracts had wide ranges of antimicrobial activity as well as contains many biologically active compounds. Especially, GC-MS analysis identified ten bioactive compounds from methanolic extract, which may serve as candidate for the discovery of novel drugs in the treatment of diverse of human UTI diseases.

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