

**TO STUDY THE ANTIMICROBIAL ACTIVITY OF VAZHAI KIZHANGU NEER AND AMURI AGAINST UTI BACTERIA**

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**ABSTRACT**

**Aim:** To study the antimicrobial activity of Vazhai kizhangu neer and Amuri against UTI bacteria.

**Methods:** The present study was done in the department of ayurveda. The antimicrobial study was performed by using well diffusion method for both samples of Amuri (Sample-A) and Vazhai kizhangu Neer (Sample-B). The following strains *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used for the study and maintained in good laboratory condition.

**Results:** The result shows that, the zone of inhibition for Amuri against common urinary pathogens are *E.coli* 16mm, *Pseudomonas aeruginosa* 12mm, *Proteus mirabilis* 13mm and *Klebsiella pneumoniae* 14mm, whereas Vazhai Kizhangu Neer, *E.coli* 15mm, *Pseudomonas aeruginosa* 10mm, and *Klebsiella* 15mm at the concentration of 2000µg.

**Conclusion:** The present anti-microbial study results revealed that, the both samples of *Amuri* and *Vazhai kizhangu neer* have been proved as a potent agent against the common urinary pathogens and concluded that the test drugs can be used for the treatment of urinary tract infection. Further studies to be needed on the yield of *Amuri* and its pharmacological actions.

**Introduction**

The process of inhibition or destroying the growth of microbes is defined as antimicrobial activity. It may be antiviral, anti-bacterial, anti-fungal. They all have different way of action through which they act to overcome the infection. Preventing the multiplication of bacteria is via the antiseptics and disinfectant agents by collapsing the bacterial cell or bacterial conjugation. The widely using method for antimicrobial activity screening is well diffusion method for plants or microbial extracts. An infection of urinary system, kidneys, ureters, bladder and urethra is known as Urinary Tract Infection (UTI). It commonly targets the women. About half of women experienced UTI at some time during their life.<sup>1</sup> Both Gram-negative and Gram-positive bacteria cause urinary tract infections, and also by certain fungi. Most urinary tract infections are caused by bacteria *E. coli*. Others include *staphylococcus*, *proteus*, *klebsiella pneumonia enterococcus and candida*. Antibiotics remain the centrepiece of treatment in acute UTI; In Siddha system also, many drugs, formulations and extracts have been used as remedy for Urinary Tract Infections (UTI). They are *Vazhai kattai neer (Amuri)*, *Vazhai kizhangu neer*, *Pani neer*, *Nanaari ooral neer*, etc. *Amuri* is signified as an elixir of life as it has the property of rejuvenation (*Kayakarpam or Kayakalpam*). Some of the name that represent *Amuri* in siddha literature, they are *Pazhcharu*, *Aagasa kangai*, *Suthasalam*, *Pugai neer*, *Kaadi neer*, *Naatha neer*, *Sivambu*, *Sivaambu*, *Kalpam*, *Sivaneer*, *Siruneer*, *Panineer*, *Ilaneer*, *Pazhaya sotru neer*, *Elumichai charu*, *Rishaba neer*, *Chandra pushpakarani neer*, *Soma neer*, *Piraineer*, *Mathipaal*, *Soma banam*, *Soma rasa panam*, etc.<sup>2</sup> *Amuri* preparation methods are concealed by Siddhars and highly preserved because of its importance in Siddha medical system. An unpublished manuscript *Kandarnaadi vaakiyam*, expresses that *Amuri* is prepared from banana tree. In this method, *Amuri* is prepared by keeping the *Guru chunnam* inside the banana tree.<sup>3</sup> In siddha literature, there are many school of thoughts in the finding and preparation of *Amuri*. According to Gunapadam Mooligai Siddha text book, *Vazhai kizhangu neer* is an extracted juice obtained from a pit made in the stump of the plantain tree. The liquid accumulate in it which is indicated for urinary tract infections, ascites, anemia and acid peptic diseases.<sup>4</sup> The present study is aimed to validate the antimicrobial activity of traditional Siddha preparation *Amuri* (an elixir from Plantain tree) and *Vazhai Kizhangu neer* against common urinary pathogens.

### **Materials and Methods**

The present study was done in the department of ayurveda.

Preparation of Guru Chunnam

Ingredients: Thurusu (Copper sulphate) - 300 gm, Sangu (Conch Shell) - 250 gm

### **Methodology**

Sangu (Conch Shell 250gm) was purified by soaking in Lemon juice for one day (24 hours) and washed with water. After drying, it was collected and subjected into traditional Pudam (calcination) process with 100 numbers of cow dung cakes. After that it was grounded well in stone mortar to become a fine powder (Sangu parpam). Purified Thurusu (Copper sulphate 300gm) was mixed with Sangu parpam and grounded well with lemon juice for 3 hrs and subjected to pudam process with 50 numbers of cow dung cakes. The Guru chunnam was collected and stored in an air tight container for the study.

Preparation of Amuri: Well grown banana tree was selected just before flowering. On the 10th lunar day a window was cut open in the neck region of the tree by cutting of small rectangular sheath layers to a depth to reach a trunk core. Three teaspoon full of Guru Chunnam (30gm) was kept inside the window and the slice was placed back and tied. The tree was irrigated. After 5 days, on the day of full moon the tree was cut off 30cm from the ground level. The cross section of the stump was scooped to create a cup like hollow. A small hole was drilled and rubber tube is inserted. An elixir was collected continuously for 5 days. The collected amuri was used for the study (Sample-A).

Preparation of Vazhai kizhangu Neer: A pit was made in the stump of the plantain tree left it for a whole night. The next day the accumulated fluid was collected and secured in a glass bottle for the study (Sample –B)

Antimicrobial Activity: The antimicrobial study was performed by using well diffusion method for both samples of Amuri (Sample-A) and Vazhai kizhangu Neer (Sample-B). The following strains *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were used for the study and maintained in good laboratory condition.<sup>5</sup>

Preparation of inoculums: The extract of study drugs were sterilized in flask, cooled to 450 – 500 °C and then poured into sterilized petriplates. The prepared MHA (Muller Hinton agar) was inoculated into the well in graded concentration (250, 500, 1000, 2000 µg/ml) using sterile syringe. The plates inoculated with bacteria will be incubated at 37°C for 24 hours. The diameter of the inhibited zone was measured in mm. The results were compared with standard drug Chloramphenicol (10µg).

Well- diffusion method: The antibacterial activities of the sample A and Sample B were carried out by well diffusion method. The concentrations of the test compounds were used at the concentration of 250, 500, 1000 and 2000 µg/ml. The microorganisms were cultured using Mueller– Hinton broth (MHB). After 24 hours, adjusted to standard sub culture dilution. The Muller Hinton Agar (MHA) medium inoculated petri dishes were cultured with diluted bacterial strain. Cylinder with 6mm diameter is created aseptically with a sterile cork borer. Each concentration was inoculated into the cylinder. Standard drug Chloramphenicol (10µg) was used as a positive reference standard to determine the sensitivity of each microbial species tested. Then the inoculated plates were incubated at 37 °C for 24 h. The clear zone around the disc's diameter was measured in millimeters as its anti-microbial.

### **Results and Discussion**

The anti-bacterial activity against common urinary pathogens has been studied for study drug Amuri (sample - A) and Vazhai kizhangu neer (sample - B). The results were tabulated in Table. no: 1, 2. Table no: 1. Anti-bacterial activity of Amuri– Zone of Inhibition (mm)

Table no: 1. Anti-bacterial activity of Amuri– Zone of Inhibition (mm)

Bacterial Strains	Concentration	Sample A (Amuri)	Chloramphenicol (10µg)
<i>Pseudomonas aeruginosa</i>	1000 µg	-	13
	2000 µg	12	
<i>Klebsiella pneumoniae</i>	1000 µg	10	13
	2000 µg	14	
<i>E.Coli</i>	1000 µg	-	18
	2000 µg	16	
<i>Proteus mirabilis</i>	1000 µg	11	15
	2000 µg	13	

The result shows that, the zone of inhibition for Amuri against common urinary pathogens are E.coli 16mm, Pseudomonas aeruginosa 12mm, Proteus mirabilis 13mm and Klebsiella pneumoniae 14mm, whereas Vazhai Kizhangu Neer, E.coli 15mm, Pseudomonas aeruginosa 10mm, and Klebsiella 15mm at the concentration of 2000µg.

Table no: 2. Anti-bacterial activity of Vazhaikizhangu neer– Zone of Inhibition (mm)

Bacterial Strains	Concentration	Sample B (Vazhaikizhangu neer)	Chloramphenicol (10µg)
<i>Pseudomonas aeruginosa</i>	1000 µg	-	12
	2000 µg	10	
<i>Klebsiella pneumoniae</i>	1000 µg	11	13
	2000 µg	15	
<i>E.Coli</i>	1000 µg	15	15
	2000 µg	15	
<i>Proteus mirabilis</i>	1000 µg	-	15
	2000 µg	-	

Nowadays resistance to the antibiotics in urinary tract infection is making trouble to control the recurrence of the disease which leads to Multi-Drug Resistance (MDR) and normal intestinal and vaginal flora dysbiosis <sup>6</sup>. Several groups of people such as diabetic patients, catheterized patients, patients with spinal cord injury, renal transplant patients are at high risk for developing UTI. A retrospective review of 70 patients with renal or peri-renal abscesses over a period of 10 years summarized that 62% were diabetic in which the isolated organisms were E. coli and Klebsiella pneumoniae <sup>7</sup>. Antibiotic resistance and their harmful effects drive the people to approach the natural remedies to maintain their immune status balanced.

In this study, traditionally prepared Amuri gained by treating with Guru chunnam exhibit higher inhibitory growth on all tested bacterial strains Pseudomonas aeruginosa, Klebsiella pneumoniae, E.Coli and Proteus mirabilis. Some studies stated that the traditional herbo mineral drug Thurusu thaivelai karukku acts against specific microbe Pseudomonas aeruginosa <sup>8</sup>. Another research study reported the good bactericidal activities of copper sulfate against Proteus vulgaris and Staphylococcus aureus <sup>9</sup>. The previous studies about the Thurusu (Copper sulphate) on antimicrobial activity strongly suggests the good antibacterial potency of Amuri (sampleA).

The consumption of Vazhai kizhangu neer / Vazhai kattai neer is the effective treatment in conventional UTI therapies. The vazhai kizhangu neer (sample B) also demonstrated its antimicrobial activity on Pseudomonas aeruginosa, Klebsiella pneumoniae, E.Coli and Proteus mirabilis.

Earlier in-vitro antimicrobial effect of crude extract of *Musa paradisiaca* Linn showed the inhibitory effect on bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and fungi *Candida albicans*, *Candida tropicalis* and *Aspergillus niger*<sup>10</sup>. Three banana plant parts (corm, pseudo stem and leaves) were evaluated for their antibacterial activity against *Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*, *Streptococcus faecal* (all Gram-positive), *Aeromonas hydrophila*, *Escherichia coli*, *Salmonella enterica* sub sp. *enterica* and *Shigella sonnei* (all Gram-negative), one fungi *Candida albicans*, showed >50% of growth inhibition<sup>11</sup>. Different *Musa* species leaf extracts of *Musa acuminata*, *Musa troglodytarum*, *Musa sapientum* and *Musa paradisiaca* were investigated for antibacterial activity against multi-drug resistant pathogens (*E. coli*, *P.aeruginosa* and *Citrobacter* species) causing nosocomial infection by agar well diffusion method<sup>12</sup>.

Scientific evidence establishes the antibiotic properties of various *Musa* species. Moreover it is easily access to the public for its cost effectiveness and ready availability. The both samples from plant extract potentially effective in suppressing microbial growth of urinary tract infection bacteria with variable potency. Sample A (*Amuri*) suppressing microbial growth of all tested bacterial strains followed by sample B (*Vazhai Kizhangu neer*) which appear to be potentially effective against four bacterial strains and less effective against *Proteus mirabilis*.

### Conclusion

The present anti-microbial study results revealed that, the both samples of *Amuri* and *Vazhai kizhangu neer* have been proved as a potent agent against the common urinary pathogens and concluded that the test drugs can be used for the treatment of urinary tract infection. Further studies to be needed on the yield of *Amuri* and its pharmacological actions.

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