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In Vitro Evaluation of Antimicrobial Susceptibility of Vettumaran Kuligai

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ABSTRACT:

This is a clinical study to evaluate the efficacy of Vettummaaran Kuligai (VMK) in comparison with an allopathic drug (Amikacin) for patients affected with mild and moderate fever. In this study, the antimicrobial effect and Minimum Inhibitory Concentration (MIC) of Seed extract with different clinical isolates and their successive comparison with commercially available antibiotics were done. The bacterial growth was inhibited by the Disk diffusion method. This *in vitro* analysis was done with various clinical isolates such as *Bacillus subtilis, Klebsiella, Proteus mirabilis, Salmonella typhi, Enterococcus, E.coli, Seeatiamarcens, Pseudomonas aeruginosa, and Staphylococcus aureus.* The best MIC values of the isolates were recorded at different dilutions of seed extract. The zone of inhibition was measured and the sensitivity and resistance of the organisms were determined. The results showed that lower dilutions of drug suspension have more susceptibility than the higher dilutions for all the clinical isolates. The results of the present study propose that lower doses of Vettumaran Kuligai will be more effective. Further exploration of the antimicrobial effects of the Vettumaran Kuligai drug against various bacterial strains should be done in detail.

Keywords: Vettumaran Kuligai, antimicrobial effects, minimum inhibitory concentration, *Bacillus subtilis, Klebsiella, Proteus mirabilis, Salmonella typhi, Enterococcus, E.coli, Seeatiamarcens, Pseudomonas aeruginosa, Staphylococcus aureus.*

INTRODUCTION:

One of the surveys conducted by the World Health Organization (WHO) reports that more than 80% of the world's population still depends upon traditional medicines for various diseases. Forced of the growing resistance of MDR microbe strains to antibiotics and other drugs, the search for alternatives is serious. There are numerous plants and natural products which have antibacterial, antifungal, and antiprotozoal effects that could be used either systemically or locally. Medicinal properties of plants have also been preferred throughout the world, due to their potent pharmacological activities, low toxicity, and economic viability, when compared with synthetic drugs. Medicinal plants are rich in a wide variety of bioactive

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secondary metabolites such as tannins, terpenoids, alkaloids, saponins, flavonoids, and phenol compounds that can produce a definite physiological action on the human body[1-3].

This study evaluates the efficacy of *Vettummaaran Kuligai* (VMK) in comparison with the allopathic drug (Amikacin) for patients affected with mild and moderate fever. In this study, the antimicrobial effect and Minimum Inhibitory Concentration (MIC) of Seed extract with different clinical isolates and their successive comparison with commercially available antibiotics.

MATERIALS AND METHODS:

The bacterial strains used for this study include *Bacillus subtilis, Klebsiella, Proteus mirabilis, Salmonella typhi, Enterococcus, E. coli, Serratia marcescens, Pseudomonas aeruginosa, and Staphylococcus aureus*. The preparation of the drug Vettumaran Kuligai is done using seed extraction. The ingredients used were Venmilagu-0.125gm, Thippili-0.125gm, Omam-0.125gm, Naabi-0.125gm, Porikaaram-0.125gm, Lingam-0.100gm, Inji-2gm, purified water-required amount. Iron, Calcium, Phosphorous, Potassium, Thiamine, Riboflavin, and Niacin were the nutritional components present in the seed extract.

Further, we have evaluated the efficacy of Vettumaran Kuligai by using antimicrobial susceptibility testing. The antimicrobial susceptibility test was performed by utilizing the minimum inhibitory concentration method. We have used five different dilutions of 10,20,40,80,160 of the seed extract in an aqueous solution (0.1 ml). Amikacin was used as a control. The antimicrobial susceptibility testing was done based on Kirby Bauer Disk Diffusion Method. The sterile Muller Hinton Agar plates were used.

Morphologically comparable inoculums were taken from an agar medium with a sterile wire loop. Then the inoculum was transferred to the test tube containing 1.5 ml sterile broth. The tubes were incubated for 2 hours at 37°C. The incubated bacterial suspension was found to be turbid. The bacterial suspension was adjusted to standard 0.5 units of McFarland turbidity. The inoculation was done by lawn or carpet culture made over the surface of the medium using a sterile cotton swab. The selected antibiotics were placed on the agar plate. The filter paper impregnated with the drug suspension at different dilutions was also placed on the agar plate. The plates were incubated at 37°C for 16 to 18 hours after which the readings were taken. The zone of inhibition was measured with the help of a zone-measuring scale and the sensitivity and resistance of the organisms were determined.

RESULTS:

The antimicrobial susceptibility test was performed with clinical isolates including *Bacillus subtilis, Klebsiella, Proteus mirabilis, Salmonella typhi, Enterococcus, E.coli, Seeatiamarcens, Pseudomonas aeruginosa, and Staphylococcus aureus* to check the antimicrobial activity of drug Vettumaran Kuligai. Amikacin was used as a control. The

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results were represented in figure 1 which indicated that lower dilutions of drug suspension have more susceptibility than the higher dilutions for all the clinical isolates.

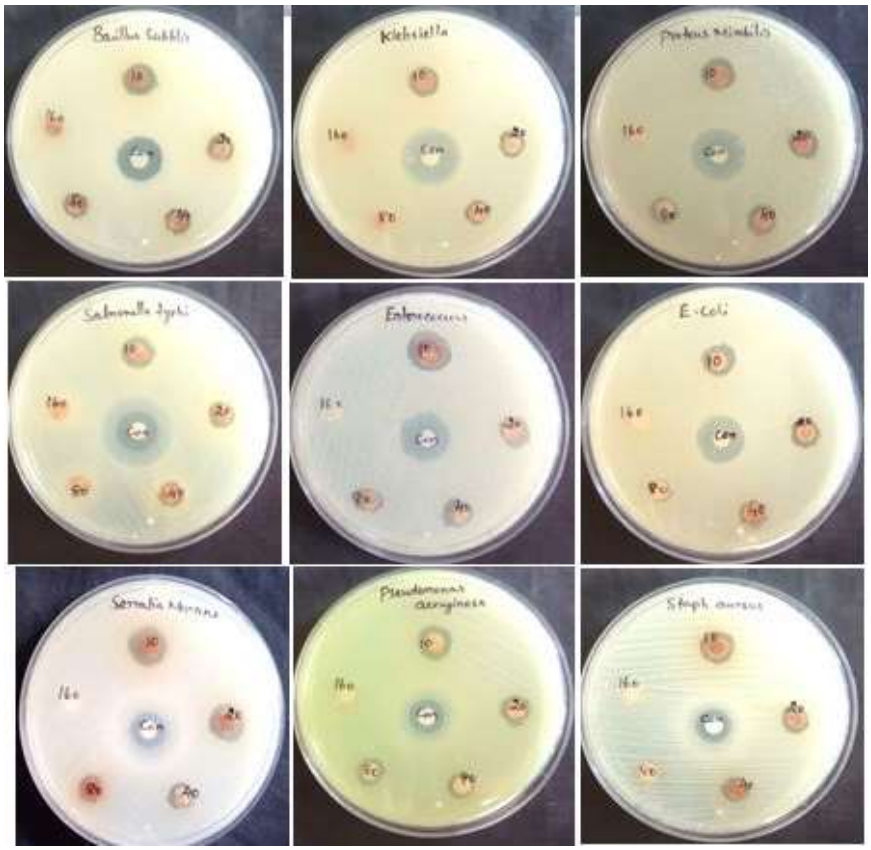

Figure 1A: Activity of multiple concentration of *Vettummaaran Kuligai* on *Bacillus subtilis, Klebsiella, Proteus mirabilis, Salmonella typhi, Enterococcus, E.coli, Seeatiamarcens, Pseudomonas aeruginosa, and Staphalococcus aureus* with Amikacin control

Table 1: Antimicrobial susceptibility pattern

Organisms	**10**	**20**	**40**	**80**	**160**	**Control (Amikacin)**
E.coli	12mm	11mm	9mm	8mm	-	16mm
Klebsiella	12mm	10mm	9mm	-	-	21mm
Pseudomonas aeruginosa	14mm	11mm	10mm	9mm	-	13mm
Salmonella typhi	13mm	10mm	8mm	-	-	15mm
Proteus mirabilis	12mm	11mm	11mm	10mm	-	19mm
Serratia marcens	16mm	14mm	11mm	9mm	-	14mm
Staph. aureus	13mm	11mm	10mm	-	-	14mm
Bacillus Subitilis	13mm	11mm	9mm	8mm	-	16mm
Enterococcus	12mm	11mm	10mm	9mm	-	16mm

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The result shown in table 1 demonstrated that the lower dilution of drug suspension shows more susceptibility than the higher dilution for all the clinical isolates. The nosocomial pathogen like *Serratia marcens* is found to be more susceptible at lower dilution of drug suspension (Table 1).

DISCUSSION:

The number of foodborne pathogens has dramatically increased in recent years[4,5]. Due to the diminishing effectiveness of antimicrobial agents and the sluggish development of new medications and antibiotics, current treatments are limited[6,7]. There are no effective treatments for these infections in humans, animals, or food. Therefore, it is crucial to create alternative antimicrobial agents for protecting human health, food safety, and animal health.

Bacillus subtillis is a Gram-positive bacterium that forms heat-resistant dormant spores, which are commonly found in soil. *Klebsiella* species are naturally present in the soil and about 40% of strains help in nitrogen fixation in anaerobic conditions. *Klebsiella* species is a Gram-negative and non-motile organism. They get transmitted from person to person directly, even when someone with contaminated and uncleaned hands touches an infected wound. Infections after intravenous and urinary catheterization, infections complicating wounds, and other infections are frequently caused by *Klebsiella* and *Serratia* species. Nosocomial infections of the lower respiratory tract, surgical wounds, and urinary tract are frequently brought on by Proteus species. Proteus species produce bacteremia less commonly, most frequently in older people. *Proteus mirabilis* is a rod-shaped Gram-negative bacterium. They do not live or grow in the presence of oxygen and become anaerobic.

Salmonella typhi is a rod-shaped Gram-negative bacillus. It's a flagellated bacterium. The only reservoir is the human body. *Enterococcus* is facultative anaerobic cocci in short and medium chains. They are a common cause of UTI, bacteremia, and infective endocarditis and rarely cause intra-abdominal infections and meningitis. *Escherichia coli* are a group of Gram-negative bacteria. It is a type of microorganism that commonly resides in the intestines of humans and animals. Intestinal infections can cause diarrhea and severe abdominal ailments. *E Coli* is a significant gastrointestinal pathogen, especially in underdeveloped nations.

Serratia marcens is an opportunistic, Gram-negative nosocomial pathogen. *Pseudomonas aeruginosa* is a rod-shaped Gram negative bacterium. They induce diseases and infections in plants and humans. *Pseudomonas aeruginosa* is a multidrug-resistant pathogen that has medical importance. They are recognized for their ubiquity and antibiotic-resistance mechanisms. *Staphylococcus aureus* is very hazardous to all staphylococcal bacteria that are Gram-positive; sphere-shaped bacteria often cause skin infections. *Streptococci* were the major nosocomial pathogens. The etiology of nosocomial infections has altered during past decades[8,9].

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Public health issues and concerns about the safety of traditional medicinal products are most widely recognized as their use throughout the world increases and a large number of new products are released into the market. Although many traditional herbal treatments have not been researched and are still in use, some of them show intriguing promise and are widely utilized. This makes it more difficult to identify the safest and most effective treatments and to encourage the sensible use of them because we know very little about their potential negative effects[10]. It is also widely known that the majority of herbal medicine's safety is further jeopardized by a lack of effective quality standards, poor labelling, and a lack of pertinent patient information[11].

Therefore, it is now crucial to provide the general public, including healthcare professionals, with sufficient information to help them better comprehend the risks involved with using these products and to ensure that all medications are secure and of the right caliber.

CONCLUSION:

The findings of the present study suggest that lower doses of Vettumaran Kuligai will be more effective against various bacterial strains. Further exploration of the antimicrobial effects of the Vettumaran Kuligai drug against various bacterial strains should be studied in detail. However, the results from the present study confirm the clinical utility of the Vettumaran Kuligai drug at the recommended dose. The relevant regulatory bodies on the need for effectiveness, proper public health protection, safety promotion, and certain significant problems related to effectively monitor the safety of these traditional treatments should consider and addressed in future research.

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