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Antimicrobial Activity of Siddha Formulations against Uropathogens

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Abstract

Urinary tract infections (UTI) is one of the most prevalent health problem caused by a number of pathogens such as E.coli, Klebsiella pneumoniae, Enterococcus faecalis, Proteus mirabilis, and Staphylococcus saprophyticus. It occurs when the bacteria infects the urinary tract or parts of the urinary tract which may even lead to urinary bladder infections as a complication. Its predisposing factors are pregnancy, previous UTI infections, age, sexual activity, poor hygiene and so on. There is a high incidence of these Urinary Tract Infections irrespective of the socio-economic groups and eventually has augmented the economic burden in families. It is one of the commonly occurring healthcare associated infections and it has been estimated that nearly 13,000 deaths are associated with UTI. Besides, there are many antibiotics prescribed for the treatment of UTI but the cause for concern is the emergence of drug resistant strains. These drug resistant strains continue to threaten our ability to treat even common illnesses. Hence, an alternative system of medicine is an essential need at the current day global scenario. Siddha, one of the ancient systems of Indian Traditional Medicine (ITM) consists of varied number of medicines for treating UTI. Siddha formulations that are chosen for the current study are Nandukkal Parpam, Karkaraichi mathirai, Rasagandi Mezhugu, Neermulli Kudineer chooranam and Sirupeelai Kudineer chooranam. Antimicrobial sensitivity test was performed using Kirby Bauer disc diffusion method and the results showed that among all the 5 formulations tested, 3 of them showed significant antimicrobial activity against all the 5 tested pathogens. Others showed considerable activity against Pseudomonas, Staphylococci and S.typhi. Hence, this study suggests a rationale in choosing siddha formulations for UTI infections.

Keywords: Urinary Tract Infections (UTI), drug resistant strains, Indian Traditional Medicine (ITM), Siddha medicine, Nandukkal parpam, Rasagandi Mezhugu, Kirby Bauer disc diffusion method

Introduction

One of the most common healthcare associated infections is Urinary Tract Infections (UTI) which affects more than 150 million people every year. It is one of the prevalent bacterial infections and has become lethal to both small and old aged group of people. Recurrent UTI infections may also lead to serious complications like pyelonephritis, renal damage, pre-term birth, etc. UTI may be distinguished as Complicated UTIs and Uncomplicated UTIs. Complicated UTIs refers to one caused by compromised immunity of the host like urinary obstruction, immunosuppression, pregnancy, transplantation, renal failure, use of catheters, stones, etc., while Uncomplicated UTIs occurs in people who are if not healthy with no known urinary tract malfunctions [1].

Prevalence of UTI:

UTI is the most prevalent cause of outpatient infections and its incidence increases with age. It is reported in about 20% of women over 65 years of age while 11% in the rest of the other population. Once affected, 27% of women report the recurrence of the disease within the next 6 months of time. Healthcare associated UTIs (HAUTIs) are reported to be the largest of the Healthcare associated Infections worldwide accounting to about 24% in developing countries. Such a high incidence of recurrence of UTI causes anxiety and stress in the affected people. Furthermore, they also cause an economic burden on the families they live in. Hence, appropriate measures have to be taken to decrease the socio economic burden as well as to ensure the physical and mental wellbeing of individuals [2].

Management of UTI & Drug Resistance strains:

The uropathogens that are mainly associated with UTI are E.coli, Staphylococcus saprophyticus, Klebsiella, Proteus, Pseudomonas, Salmonella and Enterococci. Many UTIs are treated with appropriate oral antibiotics to these uropathogen. Patients with predisposing factors of existing or recurrent UTI, urological processes or long term catheterisation, recent antibiotics show a variety of these uropathogens as mixed cultures in their urine samples. Hence besides oral antibiotic therapy, hospitalization may be necessary which solely depends on the symptoms of the patients. But when the drug resistant strains emerge, there is uncertainity in oral antibiotic therapy. E.coli and Klebsiella has been found to have nearly 20% of resistance rate to the commonly used antibiotics which Enterobacter also is found to be resistant to them. For these reasons, it is indispensable to have an alternate therapy to antibiotics for treating these uropathogens [3]. Studies also show that there is more prevalence of MRSA to antibiotics like vancomycin and erythromycin and a few to oxacillin, gentamicin and erythromycin too. Hence additional studies are required to overcome these drug resistant strains of bacteria [4].

Siddha Formulations for UTI:

Traditional systems of medicine are the greatest assets of human society combating different diseases even in the modern day world. A large number of the population are dependent on the plant based traditional medicines for various illnesses as they are not only effective as therapeutics but also exempt from causing severe side effects to the patients. In this perspective, Siddha, one of the Indian Traditional Medicine system (ITM), has received global attention over the expensive, invasive treatments with stern side effects. Siddha system of medicine contains polyherbal and herbomineral formulations with various combinations of herbs, plant, animal products and also minerals in the preparation of medicines. Many forms of Siddha medicines are available like Chooranam, Chenduram, parpam, Mezhugu, Thailam, Mathirai, Chunnam and so on. The present study has focused on describing the efficacy of chosen Siddha formulations against Uropathogens.

The drugs which are commonly used in outpatient duty and currently in clinical practise were chosen for the study to generate evidence, based on prior clinical experience. Five Siddha formulations were chosen for the study namely Nandukkal Parpam, Rasagandi Mezhugu, Karakaraichi mathirai, Neermulli Kudineer Chooranam and Sirupeelai Kudineer Chooranam to be tested against five Uropathogens- E.coli, Staphylococcus aureus, Pseudomonas, Klebsiella and Salmonella typhi. The reference for the Indications of these medicines was obtained from Siddha text Siddha Vaithiya Thirattu by Dr. Kuppusamy Mudaliar and Dr. Uthamarayan and Formulary of Siddha Medicine-1st edition, 1956. The dosage form and indications of the medicines are listed in Table 1. and in Figure 1.

Table 1. Dosage forms and Indications of chosen Siddha Formulations

S.NO.	NAME OF THE FORMULATION	DOSAGE FORM	INDICATIONS
01.	Nandukkal Parpam	Powder	Inflammation of Urogenital tract, urinary obstruction
02.	Rasagandi Mezhugu	Capsule	Urogenital cancer, Urogenital calculi, Urogenital abscess
03.	Karkaraichi Mathirai	Tablet	Renal calculi
04.	Neermulli Kudineer Chooranam	Powder	Edema, ascitis, Retention

			of urine, diuretic
05.	Sirupeelai Kudineer Chooranam	Powder	Renal calculi, retention of urine, burning sensation during micturition

Figure 1. Dosage forms of chosen Siddha Formulations

Aim & Objective:

The primary objective of the study is to determine the antimicrobial activity of the chosen Siddha formulations Nandukkal parpam, Karkaraichi Mathirai, Rasagandi Mezhugu, Neermulli Kudineer Chooranam and Siruapeelai Kudineer Chooranam gainst the five test uropathogens- E.coli, Staphylococcus aureus, Pseudomonas, Klebsiella and Salmonella typhi.

Materials and Methods:

Different methods are used in conducting the test like the Kirby Bauer disk diffusion method, well diffusion method, E-Test, broth dilution method, and so on. The method adopted for this study is Kirby Bauer disk diffusion method [5]. The media that were used are Mueller Hinton Agar (MHA), nutrient agar (NA) and nutrient broth (NB). NA and NB were used for culturing the isolates and MHA was used for estimating the antimicrobial assay [6]. Other materials that were used are Autoclave, Incubator, Zone reader, DMSO, Well maker, Micropipette, Sterile tips and glasswares. The stock cultures were obtained from SGS laboratories, Chennai. Culture media and sterile empty discs were obtained from HiMedia Laboratories.

Extract Preparation

Authenticated drugs were procured from a GMP certified Siddha drug Manufacturing company. Ethanol extraction of the siddha formulations were prepared by adding 1gm of the drug in 10ml of ethanol to obtain a 10% concentration of the medicine. Similarly 20% concentration of the extract was also prepared. The solution was subjected to frequent shaking and filtered after 24 hours to obtain the extract. (Figure. 2)

Figure 2. Ethanol extracts of the Siddha formulations

Culture Media Preparation:

For preparation of bacterial cultures, 100ml of Nutrient Agar was prepared, sterilised and poured onto 5 petriplates of about 20ml in each plate. The organisms were subcultured onto these plates from the stock culture and then used for the analysis. 50ml of Nutrient broth was prepared in 5 separate conical flasks each for 5 organisms and were labelled accordingly. The media were sterilized by autoclaving at 121˚C for 15 minutes. Once sterilised and allowed to cool, bacterial cultures were inoculated into the nutrient broth for obtaining liquid cultures of the organisms and incubated at 37˚C overnight. The next day, 24 hour culture of all the test organisms was obtained.

Antimicrobial sensitivity testing:

Kirby Bauer disc diffusion method was used for analysing the antimicrobial activity of the chosen drugs [5]. 100ml of Mueller Hinton Agar was prepared, autoclaved and poured into 10 petridishes to test for all the 5 organisms in both 10% and 20% concentration of the chosen drugs. The bacterial cultures were cultured on the MHA plate by spread plate method. The plates were labelled accordingly. Sterile empty discs procured were used for the disc diffusion method. All the 5 extracts were placed on a single plate and a negative control was included in each plate without any medicinal extract. The plates were then incubated overnight at 37˚C.

Determination of Zones of Inhibition:

The zones of inhibition of the extracts were determined using a zone reader and were marked in millimeter (mm). The extract which showed major zone of inhibition was considered to have significant antibacterial

activity, those with moderate zone was considered to have moderate activity while those that did not have any zone of inhibition was considered to have no activity on the organisms.

Results:

Table 2. Zones of Inhibition of the extracts (in mm)

S.NO.	**Name of the Extracts**	**E.coli**	**S.auresu**	**Pseudomonas**	**Klebsiella**	**S.typhi**
01.	Nandukkal Parpam	--	11	--	--	--
02.	Rasagandi Mezhugu	--	12	12	--	--
03.	Karkaraichi Mathirai	8	12	12	9	12
04.	Neermulli Kudineer Chooranam	--	11	13	9	13
05.	Sirupeelai Kudineer chooranam	8	--	13	8	9

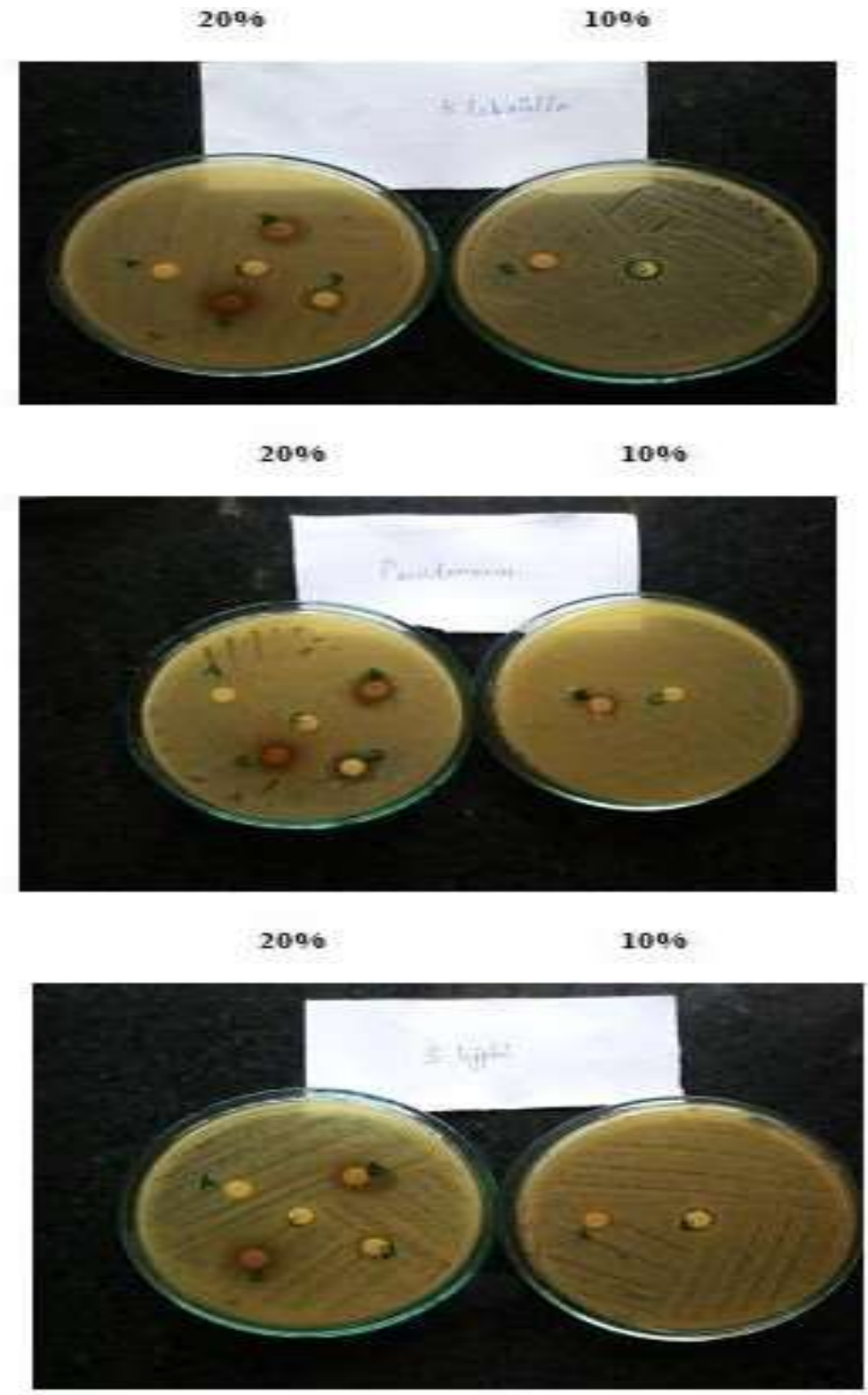

Fig. 3. Zones of inhibition

From the antimicrobial Sensitivity Test, different results for different bacteria were found. At 10 % concentration of the extracts, Karkaraichi mathirai showed significant increasing zones of inhibition towards E.coli with increasing volumes of the extract. This evidences that Karkaraichi mathirai acts considerably against E.coli.

At 20% concentration of the extracts, Nandukkal parpam showed significant activity against S. aureus

(11mm), Rasagandhi Mezhugu showed activity against S.aures (12mm) and Pseudomonas (12mm), Karkaraichi mathirai showed activity against all the tested uropathogens. Neermulli kudineer Chooranam and Sirupeelai Kudineer chooranam showed significant activity against four out of five pathogens.

Discussion:

Urinary Tract Infections (UTI) are one of the commonest infections worldwide irrespective of age groups and gender. Women are reported to be more frequently affected by recurrent UTIs than men. UTI may be of Complicated or uncomplicated types based on the physiological conditions of the individual [7]. Uropathogens have unique characteristics like production of toxins, siderophores and adhesins to gain attachment to the urinary tract. Though it is a self-limiting disease, it may recur in many cases and may also be transmitted from one person to another. Antibiotic therapy resolves the infection rapidly but accounts to the appearance of resistant strains of these uropathogens. Hence alternative strategies to combat these UTI infections are atmost necessary [8].

There are many uropathogens which causes UTI. Among them E.coli is the most prominent organism accounting to nearly 80% of the infections, Staphylococcus saprophyticus accounting to 10% and other pathogens like Klebsiella, Proteus and Enterococci to rank the next [9]. UTI are more common in children and pregnant women. They may be symptomatic or asymptomatic in children. About 7% of girls and 2% of boys of 6 years of age have symptomatic UTI. Most of the UTIs are monomicrobic, caused by single pathogen. In older children UTI may constitute symptoms like dysuria, constipation, fever, irritability and abdominal pain too [10].

Antibiotic resistance strains of uropathogens are increasingly emerging in the recent years. Uropathogenic E.coli (UPEC) is one among such resistant bacteria to commonly used antibacterial antibiotics. Important reasons for such a large increase in the worldwide distribution of antibiotic resistant strains are underuse, overuse or misuse of antibiotics. These resistant strains occur mainly because of mutation, horizontal gene transfer and their distribution [11].

Traditional systems of medicine have a long history than modern medicines. They are practised in many countries like Indian, Chinese, Iranian, Islamic etc. Siddha is one such traditional system of medicines of Indian origin, especially of the South India. In Siddha, there are various formulations that are prescribed for a number of human ailments. [12]. Siddha has a number of single herb and polyherbal formulations of plants, herbs, minerals and animal origin. Many siddha preparations have been proved to be antimicrobial [13] [14].

A number of studies have been done which focuses on the phytochemical compounds that are present in the ingredients of siddha formulations like alkalaoids, flavanoids, terpenoids and so on. It is because of these phytochemicals that these formulations exert antimicrobial activity

[16]. Among them, the medicines that are in clinical practise for UTI are the Nandukkal Parpam, Karkaraichi mathirai, Rasagandi Mezhugu, Neermulli Kudineer Chooranam and Sirupeelai Kudineer Chooranam. Hence they were chosen for the present study to be tested for their antimicrobial activity against 5 Uropathogens- E.coli, S.aureus, Pseudomonas, Klebsiella and S.typhi.

Ethanol extracts of the formulations were obtained at 10% and 20% concentrations. Culture media was prepared and the subcultures were prepared on Nutrient broth from the stock cultures. Antimicrobial susceptibility was performed using the Kirby Bauer disk diffusion method according to the protocol and the plates were incubated. After 24 hours of incubation, the zones of inhibition of the extracts were measured and the inference was made. Out of the 5 formulations that were tested against 5 uropathogens, Karkaraichi Mathirai showed antimicrobial activity against all the 5 tested pathogens while Neermulli

kudineer chooranam and Sirupeelai Kudineer Chooranam showed significant antimicrobial activity against 4 of the 5 tested pathogens. This was followed by Rasagandi Mezhugu which and Nandukkal Parpam.

To conclude, it is evident from the current study that Siddha formulations have high antimicrobial activity against important Uropathogens and are a good candidate drug of choice in the treatment of Urinary Tract Infections (UTI).

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