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Original Article

Antibacterial and Antifungal Activities of Mercury Based Traditional Siddha Herbo-Metallic Formulation *Mega Sanjeevi Mathirai* against Selected Urogenital Pathogens

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ABSTRACT

Background: Urinary Tract Infection (UTI) is referred as an infection of any part of the genitourinary tract and is more prevalent in women due to biological predisposition. *Escherichia coli* is a common causative organism. Globally, there is a concerning rise in antimicrobial resistance among UTI pathogens, which poses a public health risk. Symptoms of *Nīrccuṟukku* and *Veṭṭai noy* stated in Siddha literature are clinically correlated with UTIs. **Materials and Methods:** This study investigates *Mega Sanjeevi Mathirai (MSM)*, a Siddha herbo-metallic formulation, for its *in vitro* antibacterial and antifungal properties using Agar-Disc diffusion, Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) methods against selected urogenital pathogens. **Results:** Substantial inhibition zones against the bacterial strains of "*S. aureus, E. faecalis, E. coli and P. aeruginosa* and fungal strain of *C. albicans*", with significant sensitivity of the latter to *Mega Sanjeevi Mathirai*. MIC and MBC values against *Escherichia coli* are 41.2049 µg/mL and 74.1688 µg/mL, respectively. The test drug exhibits broad-spectrum antimicrobial activity, particularly effective against *Candida albicans*. **Conclusion:** The study concludes that *Mega Sanjeevi Mathirai* has the potential for reducing UTIs, especially in immunocompromised patients. Future research could focus on elucidating the precise mechanisms of action, molecular interactions, toxic responses and proteomic alterations in response to pathogens and this research extend to clinical trials for validating its efficacy in human subjects. Additionally, evaluating herbo-metallic formulations for potential repurposing as treatments for antimicrobial infections could pave the way for new therapeutic approaches and help combat drug resistance.

Keywords: Siddha, Urinary Tract Infection, *Nīrccuṟukku*, *Mega Sanjeevi Mathirai*, Multidrug resistance, Antimicrobial Resistance.

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INTRODUCTION

Urinary Tract Infection (UTI) is referred as an infection that significantly affects any part of the genitourinary tract. It leads to a range of symptoms such as slight discomfort during urination, bloodstream infection, severe systemic infection and potentially fatal outcomes. Structurally, it is categorized as lower and upper tract infection. Urethritis and cystitis are lower tract infections whereas upper tract infection includes conditions like pyelonephritis, prostatitis and abscesses from internal and perinephric areas. Painful and frequent urination and feel of incomplete voiding are symptoms of lower UTIs. Infections likes pyelonephritis and renal suppuration, which involve tissue invasion of the urethra and urinary bladder, are categorized as superficial or mucosal infections.[1]

Clinically, categorized as Uncomplicated (UC-UTIs) and Complicated (C-UTIs). UC-UTIs are primarily afflicted individuals without underlying health issues or structural and neurological abnormalities in the urinary tract. Lower and upper UTIs categorize these infections. Numerous risk factors contribute to cystitis, such as being female, previous UTI occurrences, sexual intercourse, infections of female external genitalia, diabetic individuals, overweight or obese and genetical

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susceptibility to UTIs. On the other hand, C-UTIs are occurs due to the impairments in the anatomical structures or host immunity. These conditions may include urinary obstructions, neurogenic urinary retention, immune system suppression, kidney malfunction, kidney transplant, pregnancy state and the existence of foreign objects likes stones, catheters, or other drainage devices.[2]

UTIs can affect both genders, but the prevalence is higher in female gender due to their shortened urethra. Especially from the 15-44 aged women are more prone. Grossly, 40% of women and 12% of men were facing UTI at least once in their lifetime. Sexual activity is the primary reason of up to 90% of bladder infections in women, particularly during the early stages of marriage, commonly referred to as "honeymoon cystitis". Other potential factors contributing to UTIs include the use of unsanitary public restrooms, the use of contraceptive devices, a family history of UTIs, urinary catheters in diabetes patients and suppressed immune system.[3]

UTIs can be caused by Gram positive and negative bacteria, as well as fungi. *Escherichia coli* stand out as the primary causative agent for both UC-UTIs and C-UTIs. In UC-UTIs, *E. coli* is the leading causative organism, followed in prevalence by "*K. pneumoniae, S. saprophyticus, E. faecalis*, Group B *Streptococcus, Proteus mirabilis, P. aeruginosa, S. aureus and Candida species*". In the case of C-UTIs, the sequence of prevalence for causative agents, with *E. coli* being the most common, includes "*Enterococcus species, K. pneumoniae, Candida species, S. aureus, P. mirabilis, P. aeruginosa* and Group B *Streptococcus*".[2]

In the Siddha system of medicine (SSM), urinary ailments are broadly categorized into two sections by the sage *Tēraiyar* in his *Tēraiyar karical.* Such as *Nīriṇaip perukkal noykaḷ* and *Nīriṇai arukkāḷ noykaḷ. Nīriṇai arukkāḷ noykaḷ* consists of six sub-categories are *Kallaṭaippu* (Urolithiasis), *Nīrccuṟukku* (Oliguria), *Nīraṭaippu* (Retension of urine), *Nīrkkaṭṭu* (Anuria), *Coṭṭunīr* (Incontinence of urine), *Veḷḷai/ Veṭṭai noy* (Urethritis/ Leucorrhea/Gonorrhea). The symptoms of *Nīrccuṟukku* and *Veḷḷai/ Veṭṭai noy* mentioned in Siddha literature *Citta maruttuvam potu* nearly related to Urinary tract infection.[4]

International guidelines on the management of UC-UTIs and pyelonephritis advocate for the use of various agents, such as "nitrofurantoin monohydrate, trimethoprim-sulfamethoxazole, fosfomycin trometamol, pivmecillinam, fluoroquinolones and beta-lactams". However, the pervasive and indiscriminate use of antibiotics is leading to a worrisome escalation in antimicrobial resistance among UTI pathogens. Bacteria produce "Extended-Spectrum Beta-Lactamases (ESBLs)", shows resistance to most antibiotics except those belonging to the carbapenem group.[5] The emergence of antibiotic resistance is evolving into a critical worldwide health issue. The escalation of multidrug resistance and pan drug resistance on a global scale presents a substantial public health challenge.[6]

Therefore, there is a need to look out for new substances from other sources like traditional systems of medicines with proven antimicrobial activity. As a result, there is a quest for enhanced antimicrobial agents derived from herbal or complementary sources, with the objective of identifying potentially valuable active phytoconstituents that can function as a foundation and model for developing novel antimicrobial agents.[7] The Siddha medical system boasts a vast array of classical texts containing numerous herbal remedies, mineral compounds, aquatic substances and animal products that may be beneficial in preventing and treating urinary tract infections. *Mega sanjeevi mathirai (MSM)* is a unique herbo-metallic formulation against chronic urinary tract infection and cystitis mentioned in Siddha formulary literature *Aṉupōka vaittiya navanītam-part VII*.[8] In the SSM; it is primarily employed to address infectious genital diseases, STDs, chronic UTIs and cystitis, particularly in patients with chronic immunocompromized.

This present work is aimed to evaluate the antibacterial and antifungal potential of Siddha herbo-metallic formulation *Mega sanjeevi mathirai (MSM)* by various *in vitro* methods.

MATERIALS AND METHODS

Ingredients of *Mega Sanjeevi Mathirai*

Ingredients[8] of the *Mega Sanjeevi Mathirai* were listed in Table 1.

Source of raw drug materials

All the ingredients were purchased from an authorized Siddha raw material store in Chennai.

Purification of raw drug materials

Liṅkam (Cinnabar-red sulfide of mercury)

A mixture of Lemon Juice, Cow's milk and *Acalypha indica* leaf juice should be thoroughly combined in equal proportions. Subsequently, this mixture is subjected to the process of *Curukku* (heating) along with *Liṅkam* (Raw Cinnabar) for purification.[9]

Raca centūram (Mercury sulphide)

The *Raca centūram* underwent a 24 hr immersion in lemon juice, after which it was removed from the container, rinsed with fresh water, dried in sunlight and subsequently stored in a container.[9]

Vīram (Mercury perchloride)

Melt 105 g (3 *palam*) of *Paṭikāram* in an iron vessel. Place 01 *palam* (35 g) of Per-chloride of mercury (*Vīram*) on it and invert *Vīram* using an iron knife. Ensure *Vīram* does not adhere to the base of the vessel, exercising caution during the inversion. Repeat this process until *Paṭikāram* is completely dried, then allow it to cool. Utilize a knife to remove the *paṭikāram* settled over *Vīram*.

Take 1½ *palam* (52 g) of *Cūṭam*, powder it and cover the *Vīram* with the powdered *Cūṭam*. Subject it to fire, followed by a cooling period.[9]

Pūram (Mercury subchloride)

Betle leaves and Black pepper were ground together to create a poultice known as *Karkam*. Subsequently, a medium-sized mud pot was filled with water and the poultice was blended into it. The raw material (*Pūram*) was then closed with a clean white cloth to prevent exposure. Using a durable twine, one end of the cloth was secured to the *Pūram*, while the other end was tied to a bamboo stick and placed horizontally on the mud pot. The cloth-encased raw material was immersed in a mixture within a pot. The pot was consistently heated until the mixture was reduced to 3/4th of its original volume. Finally, the *Pūram* was extracted from the cloth, rinsed with clean water, sun-dried and stored in a container.[9]

Table 1: **Ingredients of *Mega Sanjeevi Mathirai*.**

Sl. No.	Ingredients		Quantity
	Vernacular name	Scientific/Botanical name	
1	*Liṅkam* (Cinnabar)	Mercury sulfide	8.75 g (2.5 *Varākaṉeṭai)*
2	*Raca centūram*	Mercury sulphide	8.75 g (2.5 *Varākaṉeṭai)*
3	*Vīram*	Mercury perchloride	8.75 g (2.5 *Varākaṉeṭai)*
4	*Pūram*	Mercury subchloride	8.75 g (2.5 *Varākaṉeṭai)*
5	*Mañcaḷ kaṭukkāy tōḷ cūraṇam*	Pericarp powder of *Terminalia chebula*	35 g (10 *Varākaṉeṭai)*
6	*Elumiccam paḻaccāṟu*	Juice of *Citrus limon*	Sufficient quantity

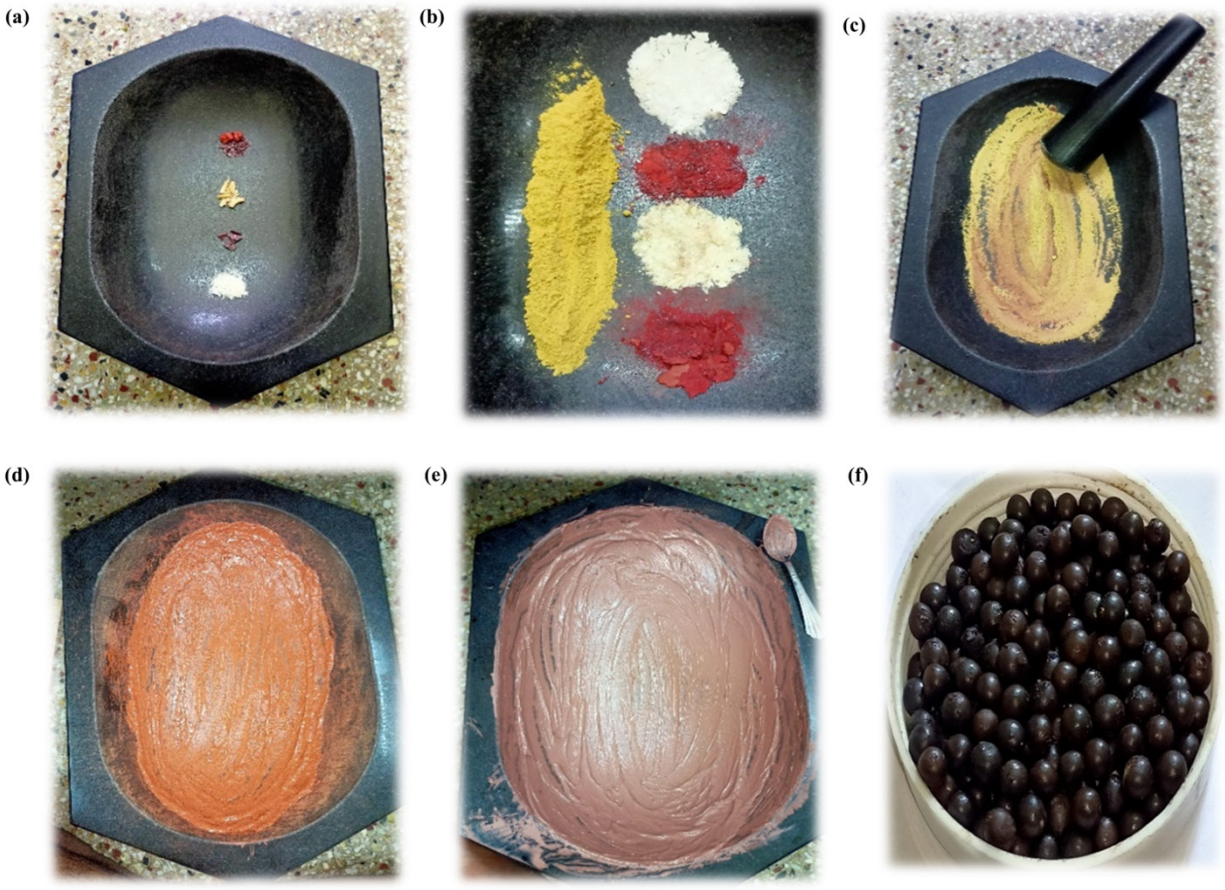

Figure 1: Preparation of *Mega Sanjeevi Mathirai*. (a) purified raw materials in stone motor (Kalvam), (b) Powdering the purified raw materials, (c) Grinding with pericarp powder of *Terminalia chebula*, (d) All the ingredients are Grinding with lemon juice, (e) After 15 hr of continuous grinding, (f) Ground outcome was made as pills (*Mega Sanjeevi Mathirai*) of 65 mg.

Standard operating procedure for preparation of *MSM*

The tablet was formulated following the established methods and procedures outlined in Siddha literature *Aṉupōka vaittiya navanītam*-Part VII. The specified four inorganic raw materials underwent individual processing with selective liquids for the purification process (*Cutti muraikal*). Following purification, the four inorganic materials, weighing 8.75 g (2.5 *Varākaneṭai*), were combined with 35 g (10 *Varākaneṭai*) pericarp powder from the *Terminalia chebula* dry fruit (Mañcaḷ kaṭukkāy tōḷ cūraṇam). The mixture was continuously ground for 15 hr (5 *Cāmam*), incorporating sufficient lime juice. Subsequently, the ground substance was formed into pills weighing 65 mg (1/2 *Kuntri edai*), which were then air-dried at room temperature. This procedure was depicted in Figure 1.[8]

Anti-Bacterial activity

Method adopted: Agar- Disc diffusion method

Principle

The antimicrobial agents within the specimens diffuse into the surrounding medium and interact with freshly seeded plates containing the target microorganisms. The resulting zones of inhibition display a uniform circular pattern due to the continuous growth of microorganisms. The diameter of these inhibition zones is measured in millimeters.[10]

Required materials

Necessary materials include Muller Hinton Agar Medium (1L), Nutrient broth (1L), sterile Whatman paper discs with a diameter of 10 mm, Streptomycin (a standard antibacterial agent with a concentration of 10 mg/mL) and cultures of test organisms adjusted to 0.5% according to the McFarland Standard, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923).[10]

Procedure

The procedure includes inoculating Petri dishes with 20 mL of Muller Hinton Agar Medium and seeding them with bacterial cultures calibrated to 0.5% of McFarland's standard for *Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus*. Sterile paper discs containing the test samples (MSM) are then placed on the plates, which are

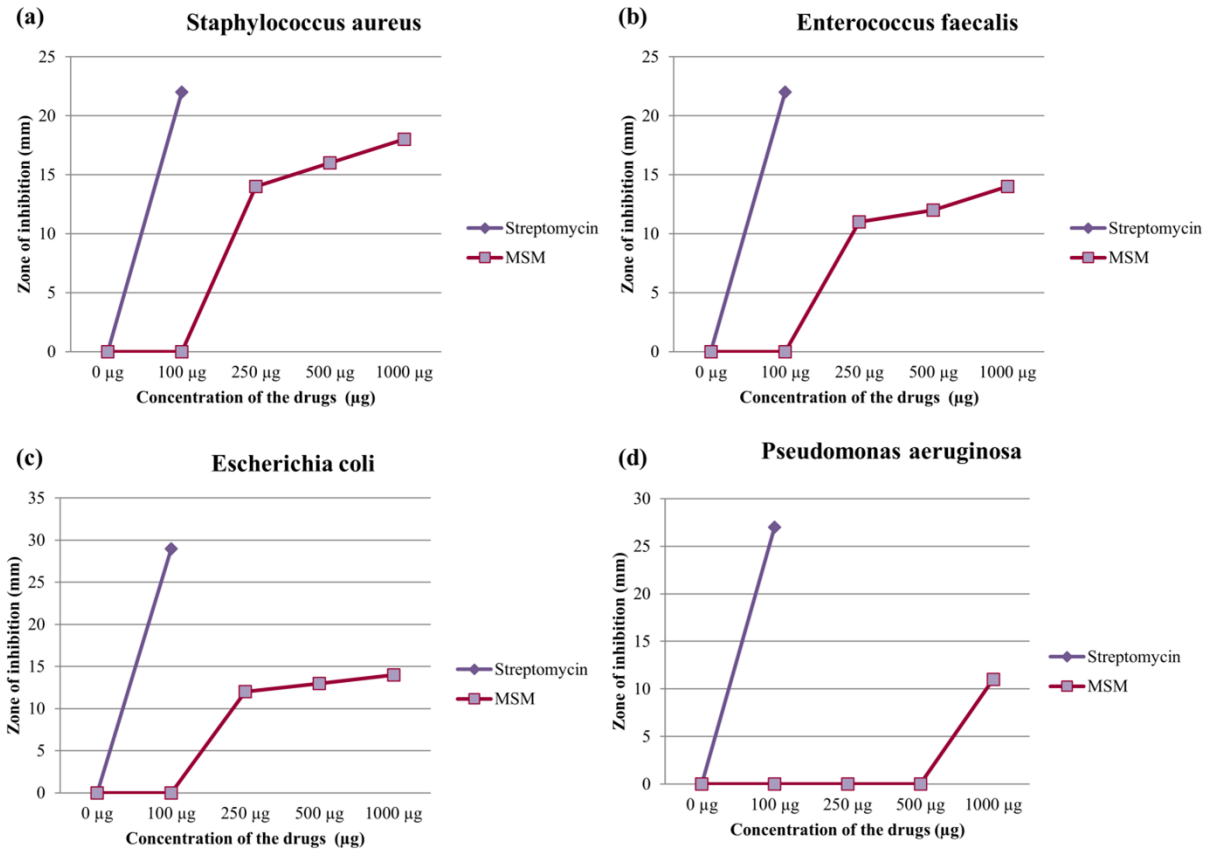

Figure 2: Zone of Inhibition (mm) measurement data of antibacterial potential of MSM; *(a) Staphylococcus aureus*, (b) *Enterococcus faecalis*, (c) *Escherichia coli* and (d) *Pseudomonas aeruginosa.*

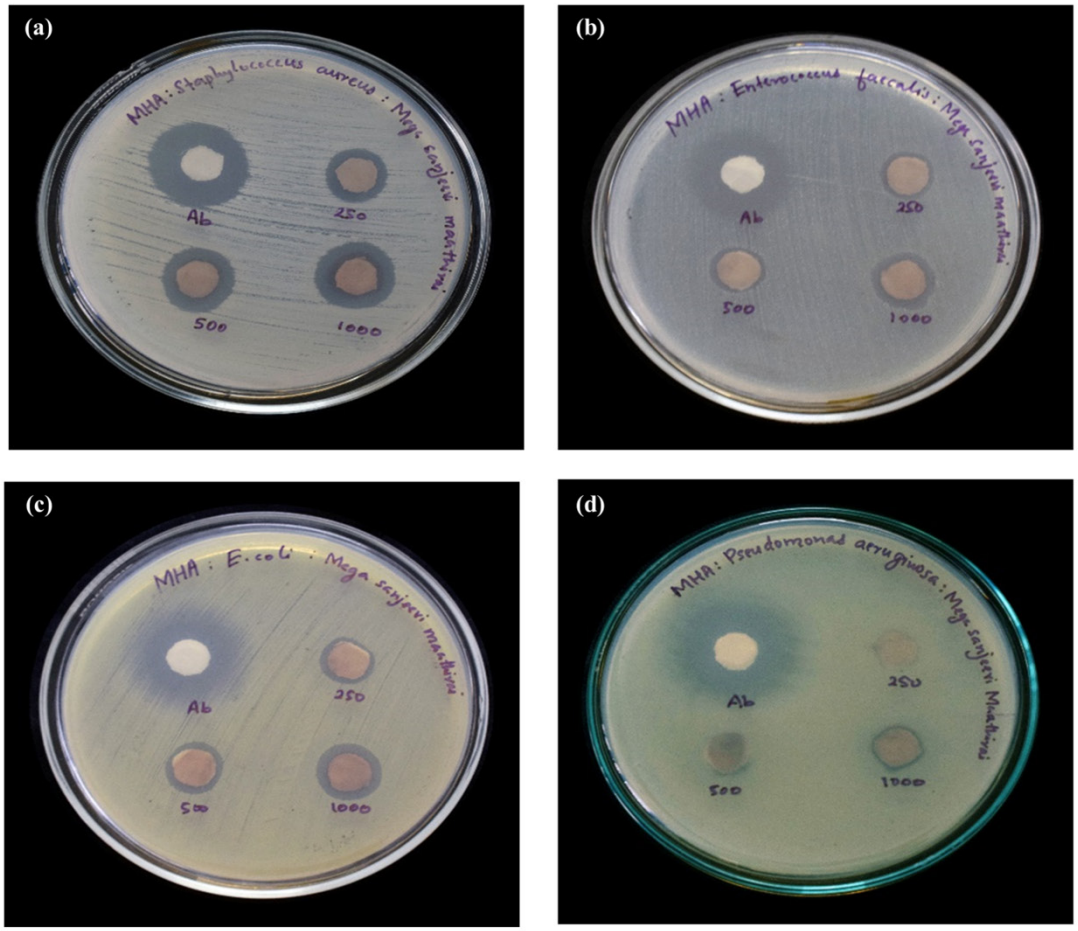

Figure 3: Anti-Bacterial activity of MSM. (a) *Staphylococcus aureus*, (b) *Enterococcus faecalis*, (c) *Escherichia coli* and (d) *Pseudomonas aeruginosa.*

incubated at 37°C for 24 hr. Antibacterial activity is determined by measuring the diameter of the inhibition zones surrounding the discs and compared to the standard antibacterial reference (Streptomycin) according to the NCCLS guidelines from 1993.[10]

Anti-fungal activity

Method adopted: Agar- Disc diffusion method

Principle

The Agar well diffusion method was employed to evaluate the antifungal activity of MSM, aiming to determine its biological significance and effectiveness. Within this method, the antifungal components present in the samples diffuse into the medium surrounding the plate, interacting with freshly seeded test organisms. The resulting inhibition zones display uniform circular patterns due to the continuous growth of fungal cultures. Measurement of the inhibition zone's diameter is expressed in millimeters.[10]

Required materials

Materials required include one liter of Potato Dextrose Agar Medium and Clotrimazole (a standard antifungal agent with a concentration of 10 mg/mL). Additionally, cultures of test organisms, specifically Candida albicans (ATCC 10231), adjusted to 0.5% according to the McFarland Standard, are needed.[10]

Procedure

In the procedure, Potato Dextrose agar plates are prepared and swabbed with overnight-grown Candida albicans fungi. Sterile paper discs containing the specific test samples (MSM) are then placed on the plates. Following overnight incubation at room temperature, the inhibition zone is assessed and compared to the standard antifungal reference (Clotrimazole) as per NCCLS guidelines from 1993.[10]

Determination of minimal inhibitory concentration

The MIC was determined using a two-fold serial dilution method.

Required materials

The initial inoculum, adjusted to a 1% McFarland Standard, consisted of *Escherichia coli* (ATCC 25922). The experiment utilized Nutrient Broth media prepared by dissolving 13g of Nutrient Broth media (HiMedia) in 1000 mL of distilled water, which was then autoclaved. Additionally, Dimethyl Sulphoxide

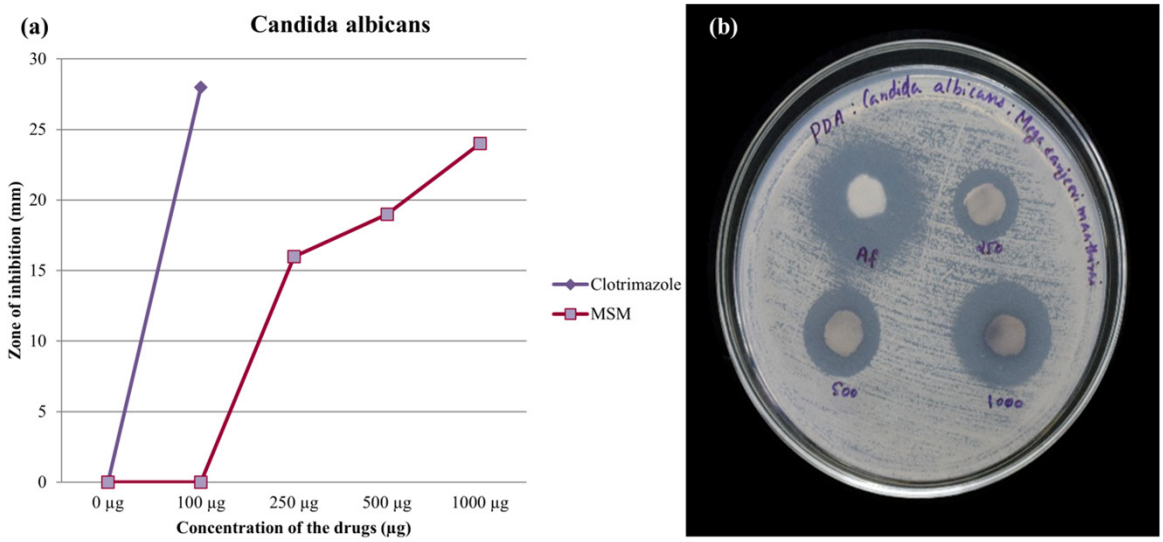

Figure 4: Anti-Fungal activity of MSM. (a) Zone of Inhibition (mm) measurement data; (b) Anti-Fungal activity of MSM against *Candida albicans.*

(DMSO) from HiMedia, a 96-well microliter plate and an ELISA plate reader (ERBA, LisaScan) were employed.[10]

Procedure

The procedure involved a broth dilution assay in a 96-well plate, where each well received 100 µL of doubly diluted conidial inoculum suspensions to a final volume of 200 µL. The sample, dissolved in DMSO at 10 mg/mL, was added in concentrations of 250 µg, 500 µg, 1000 µg, 2000 µg, 4000 µg and 8000 µg, followed by overnight incubation at room temperature. A control well contained only the organism. Growth was visually assessed and the Optical Density (OD) at 630 nm was measured using an ELISA plate reader immediately after inspection.[10] Growth inhibition at each extract dilution was determined using following formula:

$$\text{Percentage of inhibition} = (\text{OD of control} - \text{OD of test})/(\text{OD of control}) \times 100$$

Determination of minimal bactericidal concentration

Required materials

Nutrient broth, Muller Hinton agar plates, bacterial culture (*E. coli* [ATCC 25922] with growth adjusted to 1% McFarland Standard) and 96-Well Microtiter Plate.[10]

Procedure

The MBCs against *E. coli* were assessed *in vitro* for the MSM using a protocol similar to the MIC procedure. Each well received 100 µL of doubly diluted inoculum suspensions, resulting in a final volume of 200 µL, with samples added at concentrations of 62.5, 125, 250, 500 and 1000 µg. After 24 hr of incubation at room temperature, 20 µL from the 250 and 1000 µg wells were swabbed onto Muller Hinton agar plates and incubated at 37°C for 48 hr. The MBC was determined as the lowest concentration showing no growth or fewer than three colonies, indicating 99 to 99.5% killing activity.[10]

RESULTS

Anti-microbial activity (antibacterial and antifungal) of *Mega Sanjeevi Mathirai*

The antibacterial and antifungal potential of the Siddha herbo-metallic drug MSM is depicted in Figures 2-4. In the tested strains, a measurable zone of inhibition (mm) was observed, ranging from the lowest concentration (250 µg/mL) to the highest concretion (1000 µg/mL) of the MSM. The inhibition zone by MSM was found as dose-dependent across concentrations from 250 to 1000 µg/mL. Specifically, on the *S. aureus* strain, inhibition zones of 14 mm, 16 mm and 18 mm were observed; on the *E. faecalis* strain, zones of 11 mm, 12 mm and 14 mm were noted; the *E. coli* strain exhibited zones of 12 mm, 13 mm and 14 mm; *P. aeruginosa* strain displayed zones of 0 mm, 0 mm and 11 mm; and *C. albicans* strain showed zones of 16 mm, 19 mm and 24 mm at concentrations of 250, 500 and 1000 µg/mL of MSM, respectively.

Comparatively, the control Streptomycin demonstrated the maximum zone of inhibition on *S. aureus, E. faecalis, E. coli and P. aeruginosa* strains, measuring 22 mm, 22 mm, 29 mm and 27 mm, respectively. Additionally, the control Clotrimazole exhibited the highest zone of inhibition on *C. albicans* strain, measuring 28 mm.

Table 2: Minimal inhibitory concentration of MSM against *Escherichia coli*.

Concentration(µg)	Absorbance	Absorbance	Absorbance	Average	% of Inhibition
Control	0.9423	0.999	0.9982	0.9798	-
Mega Sanjeevi Mathirai					
62.5	0.2388	0.2358	0.2547	0.2431	75.84
125	0.1873	0.1817	0.1884	0.1858	81.53
250	0.0992	0.0960	0.0991	0.0981	90.25
500	0.0497	0.0551	0.0574	0.0540	94.62
1000	0.0292	0.0150	0.0177	0.0206	97.94

Table 3: Minimal bactericidal concentration of MSM against *Escherichia coli*.

Sample	Concentration (µg)	No of colony counted	CFU/mL
Streptomycin	Control	1704	85.2×10^3
Mega Sanjeevi Mathirai	250	-	-
	1000	-	-

MIC and MBC Values of *Mega Sanjeevi Mathirai* against *E. coli*

The assessed and compiled values for the MIC and MBC of MSM are presented in Tables 2 and 3 respectively. *Mega Sanjeevi Mathirai* demonstrated an MIC_{50} value of 41.2049 µg/mL against *Escherichia coli* and its MBC (IC_{90}) value, determined using ED_{50} PLUS V1.0 Software, was 74.1688 µg/mL. Figure 5 depict the MIC and MBC of MSM against *Escherichia coli*.

DISCUSSION

In this study, we employed the "Agar well diffusion method" to assess the antimicrobial efficacy of *Mega Sanjeevi Mathirai* against Gram +ve and Gram -ve bacteria as well as fungal strains in the urogenital region. The results revealed a noteworthy antimicrobial effect, encompassing both antibacterial and antifungal activities of the MSM test drug. When compared to the standard drugs (Streptomycin and Clotrimazole), MSM demonstrated significant efficacy. The antimicrobial activity varied among the diverse microorganisms examined, suggesting that the effectiveness of MSM may be contingent upon the drug's chemical composition and the membrane permeability of microbes to its chemicals and metabolic processes. The MIC and MBC values of MSM against *E. coli* were also given significant values.

E. coli commonly resides in the GIT of both humans and animals. Normally, *E. coli* forms a mutually advantageous symbiosis with its host, aiding in maintaining the balance of the gut microbial community and supporting regular intestinal function. As a commensal, *E. coli* typically resides harmlessly within the intestinal tract and rarely leads to illness. In individuals with weakened immune systems or compromised gastrointestinal barriers, even harmless commensal strains of *E. coli* can cause infections. Pathogenic strains of *E. coli* are broadly classified as either "enteric/diarrheagenic *E. coli* or extraintestinal *E. coli* (ExPEC)". Enteric/diarrheagenic *E. coli* includes six distinct pathotypes: "Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely Adherent *E. coli* (DAEC)". Among ExPEC, uropathogenic *E. coli* strains account for the majority of UTIs. Which contributes both community and hospital acquired UTIs, resulting in significant medical costs and global morbidity and mortality.[11] In the present study, 12 mm, 13 mm and 14 mm of inhibition zone was observed on *Escherichia coli* strain with various concentrations of MSM. The MIC and MBC values of MSM against *E. coli* were 41.2049 µg/mL and 74.1688 µg/mL respectively. It reveals MSM is moderately sensitive to *E. coli* strain.

Candida species, especially *C. albicans*, are significant opportunistic pathogens that cause UTIs acquired in healthcare settings. Both "*C. albicans* and non-*C. albicans* Candida (NACA)" species are natural components of the microbial flora in the mouth, digestive tract and vagina among various healthy individuals. Moreover, they colonize in the urethral opening of premenopausal women. An immune deficiency can disrupt the balance between *C. albicans*, NACA yeasts and other host normal flora. Under such circumstances, the normally commensal *Candida* yeasts can transform into opportunistic pathogenic microorganisms, leading to the development of candidal UTIs in the host. The presence of above-mentioned candida species in urine, known as candiduria, can result in both asymptomatic and symptomatic UTIs. Although UTIs caused by *C. albicans* are linked to increased morbidity, the mortality rate remains minimal. However, patients with systemic candidiasis and AIDS exhibit a high mortality rate.[12] In the current investigation, varying concentrations of MSM demonstrated inhibition zones of 16 mm, 19 mm and 24 mm against the *C. albicans* strain, which were comparable to or less than the control drug Clotrimazole (28 mm). This indicates that MSM is highly responsive to the *C.*

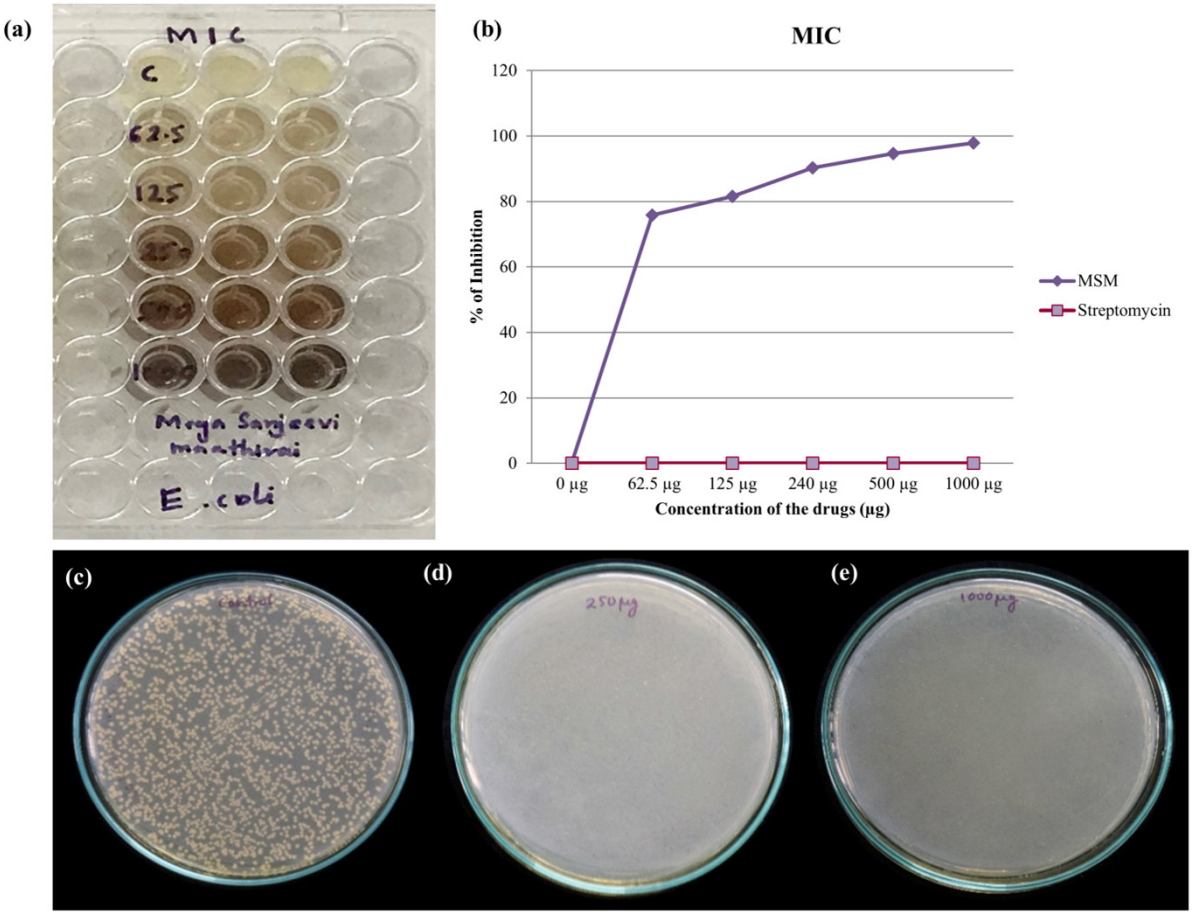

Figure 5: (a and b) MIC of MSM against *Escherichia coli*; (c) MBC of Control drug against *Escherichia coli*; (d) MBC of MSM at 250 µg Concentration against *Escherichia coli*; (e) MBC of MSM at 1000 µg Concentration against *Escherichia coli*.

albicans strain and may be beneficial in managing nosocomial UTIs and UTIs in immunocompromised patients.

UTIs caused by *S. aureus* are not frequent, accounting for only 0.5% to 1% of cases. The presence of *S. aureus* in urine samples may indicate asymptomatic colonization. *S. aureus* Bacteriuria (SABU) is characterized by the presence of *S. aureus* in urine samples, regardless of concentration (CFU/mL) and is unaffected by the presence of other identified pathogens. The main risk factor for SABU is urinary catheterization (63%-82%), followed by urinary obstruction, medical procedures, or recent hospital stays, particularly in older males.[13] In this study, varying concentrations of *Mega Sanjeevi Mathirai* (MSM) resulted in inhibition zones of 14 mm, 16 mm and 18 mm against *Staphylococcus aureus* strains, indicating a high sensitivity of *Mega Sanjeevi Mathirai* to the *S. aureus* strain.

E. faecalis, a gram-positive bacterium, has the potential to induce various nosocomial infections, with urinary tract infections being the most prevalent among them. The treatment of these infections poses significant challenges due to the drug resistance exhibited by numerous isolates of *E. faecalis*. Despite the complexities associated with these infections, there is limited understanding regarding the host and bacterial factors essential for *E. faecalis* to initiate urinary tract disease.[14] In the present study, 11 mm, 12 mm and 14 mm of inhibition zone was observed on *E. faecalis* strain with various concentrations of MSM. It reveals *Mega Sanjeevi Mathirai* is moderately sensitive to *E. faecalis* strain. *P. aeruginosa*, characterized as an opportunistic human pathogen, has the potential to induce significant UTIs. Due to its inherent high resistance to antibiotics and its capacity to acquire additional resistance during antibiotic therapy, effectively eliminating these infections poses considerable challenges.[15] In the present study, 0 mm, 0 mm and 11 mm of inhibition zone was observed on *P. aeruginosa* strain with various concentrations of MSM. It reveals *Mega Sanjeevi Mathirai* is resistant in 250 and 500 µg/mL of concentrations, despite mild sensitive in 1000 µg/mL of concentration to *E. faecalis* strain.

In vitro anti-microbial activity of *MSM* was compared with previous studies of other herbal, herbo-mineral and herbo-metalic siddha drugs. Anitha Akilan *et al.* investigated the antimicrobial activity of various herbo-mineral Siddha drugs, including *Kungiliya parpam (KP), Vengara parpam (VP), Padikara parpam (PP) and Silasaththu parpam (SP),* against *E. coli, P. aeruginosa* and *S. aureus*. The results indicated that *VP* and *PP* exhibited antibacterial activity with mild to moderate sensitivity,

while *KP* and *SP* did not demonstrate antimicrobial effects.[16] When contrasting the findings of this investigation with the aforementioned antimicrobial activity, it was observed that MSM exhibits a moderate to high sensitivity to *E. coli, P. aeruginosa* and *Staphylococcus aureus* strains compared to *KP, VP, PP* and *SP*. A study conducted by H. Nalini Sofia *et al.* on *Nandukkal parpam (NP)* demonstrated its antimicrobial properties against *E. coli* and *P. aeruginosa*, showing mild sensitivity. The drug, however, exhibited ineffectiveness against *S. aureus*.[17] In the analysis of the current study, it is evident that MSM demonstrates a moderate to high level of sensitivity against *Escherichia coli, P. aeruginosa* and *S. aureus* strains when compared to *NP*. M. Shri Saranya *et al.* studied the antibacterial activity of Siddha poly herbo-mineral drug *Gandhaga Rasayanam (GRM)*, demonstrating its antimicrobial properties against *E. coli* and *S. aureus* with mild to moderate sensitivity. However, the drug proved ineffective against *P. aeruginosa*.[18] In comparing the findings of the current study, it is evident that MSM demonstrates moderate to high sensitivity against the three strains mentioned, surpassing *GRM*.

Lalitha Sunanna et al. investigated the antimicrobial activity of the classical Siddha medicine *Seenthil Choornam (SC)*, revealing significant efficacy against *S. aureus, E. faecalis and P. aeruginosa* with mild to moderate sensitivity. However, the drug was found to be ineffective against *Escherichia coli*.[19] In comparing the findings of this study with the aforementioned antimicrobial activity, it was observed that MSM exhibits a moderate to high sensitivity against *E. coli, E. faecalis, P. aeruginosa* and *S. aureus* strains when compared to *SC*. Christian G J *et al.* studied the antimicrobial potential of *Kanagalinga Mezhugu (KLM)* against specific urogenital pathogens, demonstrating noteworthy antimicrobial effects. The results indicated high sensitivity to *Escherichia coli* and moderate to high sensitivity to *P. aeruginosa, S. aureus* and *C. albicans*.[20] In the current research, *Mega Sanjeevi Mathirai* exhibited significant antimicrobial activity, showing high sensitivity to *Candida albicans* and moderate to high sensitivity to *P. aeruginosa, S. aureus and E. coli*. A comparative analysis of *KLM* and *MSM* suggests that both are equally potent in combating urogenital pathogens.

Upon analyzing the outcomes of diverse *in vitro* antimicrobial assessments, a noteworthy variance in the antimicrobial sensitivity emerged when comparing *Mega Sanjeevi Mathirai* with other herbal, herbo-mineral and herbo-metallic Siddha drugs, particularly in relation to MSM. The results imply the potential efficacy of *Mega Sanjeevi Mathirai* in reducing urinary tract infections. This suggests that *Mega Sanjeevi Mathirai* exhibits a distinct antimicrobial profile compared to other Siddha drugs, emphasizing its potential in addressing urinary tract infections based on the observed differences in antimicrobial sensitivity.

CONCLUSION

In the present study, findings suggest that *Mega Sanjeevi Mathirai* (MSM) displayed a wide range of antimicrobial effectiveness against various organisms, with *Candida albicans* exhibiting notable sensitivity. Therefore, the test drug *Mega Sanjeevi Mathirai* has the potential to reduce UTIs, especially in immunocompromised patients. This research contributes to the ongoing efforts to find effective and sustainable solutions for managing urogenital infections, emphasizing the need for a multidisciplinary approach to bridge the gap between *in vitro* promise and clinical reality. Future research could focus on elucidating the precise mechanisms of action, molecular interactions, toxic responses and proteomic alterations in response to pathogens and this research extend to clinical trials for validating its efficacy in human subjects. Additionally, evaluating herbo-metallic formulations for potential repurposing as treatments for antimicrobial infections could pave the way for new therapeutic approaches and help combat drug resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AIDS: Acquired Immunodeficiency Syndrome; **ATCC:** American Type Culture Collection; **AMR:** Antimicrobial Resistance; **C-UTIs:** Complicated Urinary Tract Infections; **DAEC:** Diffusely Adherent *Escherichia coli;* **DMSO:** Dimethyl Sulphoxide; **ED_{50}:** Effective Dose 50; **EHEC:** Enterohemorrhagic *Escherichia coli;* **EIEC:** Enteroinvasive *Escherichia coli;* **ELISA:** Enzyme-Linked Immunosorbent Assay; **ESBLs:** Extended-Spectrum Beta-Lactamases; **ETEC:** Enterotoxigenic *Escherichia coli;* **EAEC:** Enteroaggregative *Escherichia coli;* **GIT:** Gastrointestinal Tract; **IC_{90}:** Inhibitory Concentration 90; **MBC:** Minimal Bactericidal Concentration; **MHA:** Mueller-Hinton Agar; **MIC:** Minimal Inhibitory Concentration; **NACA:** Non *Candida albicans*; **NCCLS:** National Committee for Clinical Laboratory Standards; **OD:** Optical Density; **SSM:** Siddha System of Medicine; **SABU:** *Staphylococcus aureus* Bacteriuria; **UC-UTIs:** Uncomplicated Urinary Tract Infections; **UTI:** Urinary Tract Infection.

AUTHOR CONTRIBUTIONS

Concept, medicine preparation, data analysis, interpretation: Siva Annamalai and Anbarasan Balasubramaniyan. Literature collection and manuscript writing: Siva Annamalai. Final

manuscript review and approval: Siva Annamalai, Jayaveeran Thavaseelan, Deepa Ravichandran, Sathya Rathish Mohana Jeevanath, Suresh Ramasamy, Elamathi Srinivasan and Anbarasan Balasubramaniyan.

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