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Anti-Bacterial Potential of Siddha Herbo-Mineral Formulation *Linga Chenduram*: An In-Vitro Study

Thiruvancheeswaran Soruban*[1], Sathiyaseelan V[2]

[1]Postgraduate Institute of Indigenous Medicine, University of Colombo, Sri Lanka
[2] Faculty of Siddha Medicine, University of Jaffna, Sri Lanka

ABSTRACT

Many existing antibiotics have limitations regarding their effectiveness against various pathogens and often cause adverse effects. Overuse of these antibiotics has led to the emergence of drug-resistant microorganisms. The Siddha system of medicine offers promising potential for combating these resistant pathogens. *Linga Chenduram* (LC), a traditional herbo-mineral preparation mentioned in the ancient Siddha text *Anuboga Vaithiya Navanitham*, was the focus of this study. The aim of this study was to screen the anti - bacterial potential of Siddha herbo-mineral formulation LC. Anti-bacterial activity of the sample was tested for *E.coli* (ATCC 25922*), Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923) to determine the diameter of inhibition zone (DIZ), minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC). The study results demonstrate that a concentration of 1000 µg/mL of LC effectively inhibited the growth of all tested organisms. The minimum bactericidal concentration (MBC) was determined to be 250 µg/mL. At this concentration, the remaining colony counts were as follows: *E. coli* (285 colonies, equivalent to 14.2 x 10^3 CFU/mL), *Pseudomonas aeruginosa* (96 colonies, equivalent to 4.8 x 10^3 CFU/mL), and *Staphylococcus aureus* (33 colonies, equivalent to 1.65 x 10^3 CFU/mL). The minimum inhibitory concentration (MIC) at which 50% of the bacteria were inhibited (MIC50) was 405.584 µg/mL, 459.61 µg/mL, and 515.575 µg/mL for *E. coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively. Based on these results, it can be concluded that *Linga Chenduram* (LC) exhibits promising antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*. This suggests its potential as a natural alternative or adjunct therapy for infections caused by these pathogens

Keywords: Anti-bacterial, E-coli, *Linga Chenduram, Pseudomonas aeruginosa*, *Staphylococcus aureus*

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Corresponding Author: sorruthiru@gmail.com
Orchid ID: 0000-0001-6122-4241

Introduction

Infectious diseases remain a major global health concern, contributing to 41% of the worldwide healthcare burden (Hemeg *et al.,* 2020; Noah & Fidas, 2000). A key driver of this issue is the growing prevalence of bacterial resistance to current antibiotics (Prestinaci et al., 2015; Cassini *et al.,* 2019). Recent research by Murray et al. estimated that up to 1.27 million deaths were associated with bacterial antimicrobial resistance (AMR) in 2019 alone (Murray, *et al.,* 2022) Mineral based preparations are widely used as antimicrobial agents for centuries (Waters *et al.,* 2023; Sharma *et al.,* 2022). However, the efficacy and mechanisms of action of mineral based preparations are uncertain due to the insufficient of antimicrobial studies. The Siddha system medicine is one of the oldest medical systems in the Southern India, Northern and Eastern Sri Lanka (Soruban *et al.,* 2022). This system boasts a vast pharmacopoeia encompassing plant, animal, and mineral-based remedies. The use of mineral drugs, particularly before and after the era of Bogar, was well-established among Siddha practitioners (Sathiyarajeswaran *et al.,* 2009). Silver, gold, zinc, copper, and other metals, renowned for their antimicrobial properties in modern medicine, have been employed as life-saving treatments for infectious diseases for millennia within the Siddha system (Michael *et al.,* 2011). In the Siddha system, taste plays a crucial role in drug selection, combination, and treatment. According to Siddha Taste Theory, bitter taste is believed to help destroy microorganisms (Sivakkumar *et al.,* 2016). The therapeutic values of some Siddha formulations have been well documented earlier, but a huge number of them remain unexplored in terms of safety and efficacy. Among this, *Linga Chenduram* (LC) *is* one of the internal preparations mentioned in *Anuboga Vaithiya Navaneetham* indicated for *Mega Noi* (Sexually Transmitted Disease), *Kiranthi* (Syphilis), *Pun* (Wound)*, Purai* (Pus), *Karuppai Puzhukal* (Uterine Infection)*, Alkuzhl Puttu (Cervical Carcinoma) and Nunakkai Kiranthi* (Syphilitic Tumour) (Hakkim,1995). The aim of this study was to screen the anti - bacterial potential of Siddha herbo-mineral formulation LC.

Objectives

The objective of this study was to evaluate the antibacterial potential of the Siddha herbo-mineral formulation LC by testing its activity against selected bacterial pathogens. Specifically, the study aimed to compare the antibacterial effectiveness of LC against *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Staphylococcus aureus* (ATCC 25923). The investigation focused on determining key antibacterial parameters such as the diameter of inhibition zone (DIZ), minimum inhibitory concentration (MIC), and

minimum bactericidal concentration (MBC). Through this analysis, the study sought to assess and compare the potency of LC in inhibiting and eliminating different bacterial strains, thereby establishing its potential as a natural antibacterial agent.

Materials and Methods

Test Organisms

The following standard bacterial strains were used in this study

E coli (ATCC 25922)

Pseudomonas aeruginosa (ATCC 27853)

Staphylococcus aureus (ATCC 25923)

Determination of the Zone of Inhibition

Organisms were placed on Mueller Hinton Agar medium plates and 10mm wells were bored. Different doses of LC (presumably the experimental compound) and streptomycin (a positive control) were added to the wells. The plates were incubated at 37°C for 24 hours. Each sample was tested in triplicates and antibacterial activity was evaluated by measuring and recorded the zones of inhibition in mm (CDC *et al.,* 2019).

Determination of Minimal Inhibitory Concentration (MIC)

Organism placed in 96 well cultured plates had been compared to similar plates where different doses of LC had been subjected and visual inspection is done by measuring the optical density (OD) at 630nm using an ELISA plate reader (Balouiri *et al.,* 2016).

Percentage of inhibition = (OD of control - OD of test)/ (OD of control) × 100

Determination of Minimal Bactericidal Concentration (MBC)

Organism placed in 96 well cultured plates had been compared to similar plates where different doses of LC had been subjected and incubated for 24 hours then swabbed onto potato dextrose agar plates; incubated at 37˚c for 48 hours and observed for colony forming units (Balouiri *et al.,* 2016).

Preparation of drug

Purified *Lingam* (Cinnabar)	- 17.5g (5*Varaganedai)*
Thirugukalli Latex (*Euphorbia tortilis*)	- Sufficient
Utthamani flowers (*Pergularia daemia*)	- 70g (2 *Palam*)

Vellaierukkam flowers (*Calotropis procera)* - 70g (2 *Palam*)

Purified *Lingam* (Cinnabar) was measured (17.5g) and made into powder form with mortar and pestle. Then *Euphorbia tortilis* (*Thirugukalli*) latex (250ml) was poured into it and ground well by stone mortar and pestle for 12 hours (4 *Saamam*). The mixture of *Lingam* was then made into small disc (*villai*) and spread in a suitable pot for drying in sun light. Flowers of *Pergularia daemia* (*Utthamakani*) and *Calotropis procera* (*Vellarukkam*) were ground together and made into paste (*Karkam*). Dried disc of *Lingam* was covered with prepared *karkam* then placed into pot with lid and sealed with clay smeared cloth (*Seelai mann*). Weight of clay pot with lid containing mixture was measured. Then it was subjected into incineration process (*Pudam*) by cow dung cake (4 times weight of the measured clay pot weight (660g). After the incineration process clay pot was allowed to cool itself. Processed medicine was taken from the clay pot and ground into fine powder.

Statistical analysis

The determination of minimal inhibitory concentration was calculated as means ± SD. The significance was evaluated by analysis of variance (ANOVA) using Microsoft Excel program and Dunnett's test were performed to analyse data. Significant differences in the data were established at the 0.1% level of significance

Results and Discussion

Determination of the zone of inhibition

Table 1: Determination of the zone of inhibition

Concentration (µg/mL)	Zone of inhibition (mm)		
	E coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Streptomycin 100µg/mL	31	30	27
LC 250 µg/mL	Nil	Nil	Nil
LC 500 µg/mL	Nil	Nil	Nil
LC 1000 µg/mL	11	11	11

According to the Table 1 results, standard drug streptomycin 100µg/mL response to all the pathogens. Sample drug LC 250µg/mL and 500µg/mL are not response to the pathogens and sample drug LC 1000µg/mL response to all the three pathogens, measurement is 11mm. Even though this zone of inhibition measurement is lower than standard drug zone of inhibition measurement.

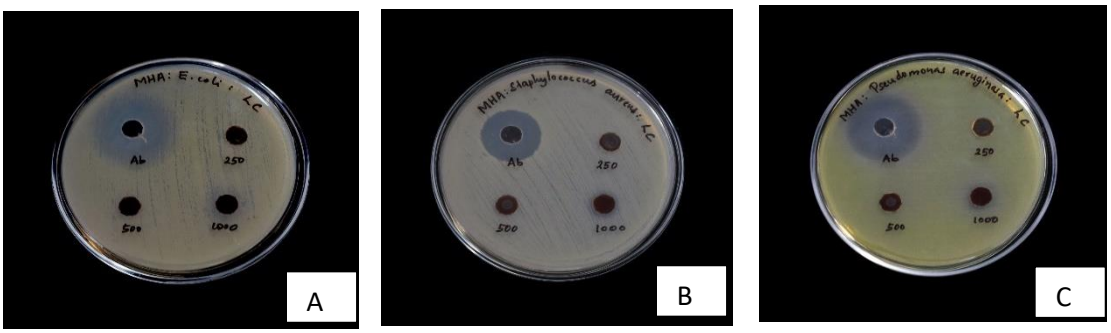

Figure 1: Images of the Zone of Inhibition against various pathogens

Figure.1 shown zone of inhibition of different organisms against streptomycin and different dosage of LC. Image A shows zone of inhibition against *E.coli*, Image B shown zone of inhibition against *Staphylococcus aureus* and Image C shown zone of inhibition against *Pseudomonas aeruginosa.*

Minimal inhibitory concentration

Minimal inhibitory concentration experiment analysed to different concentrations of LC 62.5µg/mL, 125 µg/mL, 250µg/mL, 500µg/mL and 1000µg/mL against *E.coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* All experiments were done in triplicates and results represented as Mean+/- SE. One-way ANOVA and Dunnetts test were performed to analyse data. Significant differences in the data were established at the 0.1% level of significance compared to control group.

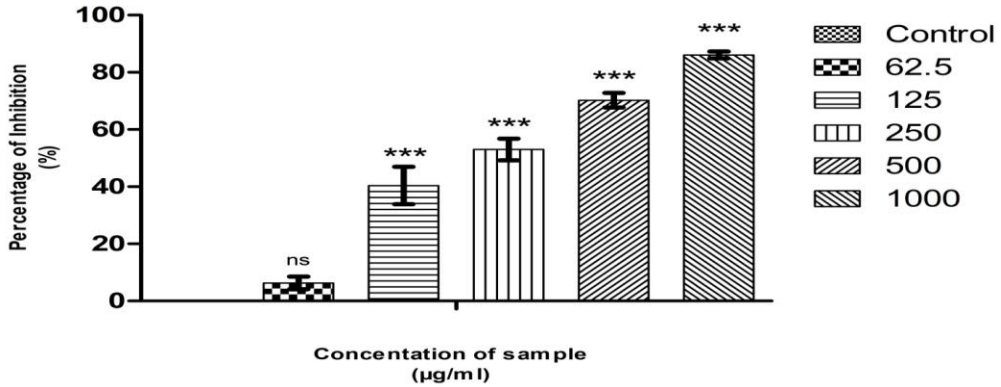

Figure 2: Graphical representation depicting the MIC of sample against *E coli*

Table 2: Determination of minimal inhibitory concentration against E coli

	% inhibition 1	*% inhibition 2*	*% inhibition 3*	*Average*	*Std*	*Std error*
Control	*0*	*0*	*0*	*0*	*0*	*0*
62.5 µg/mL	2.65436	10.1841	6.15993	6.33281	3.76786	1.25595
125 µg/mL	29.573	52.1937	39.4179	40.3949	11.3419	3.78065
250 µg/mL	46.4801	59.5817	52.9528	53.0049	6.55097	2.18366
500 µg/mL	65.3491	73.6758	71.7152	70.2467	4.35326	1.45109
1000 µg/mL	83.9008	88.0655	86.1825	86.0496	2.08554	0.69518

Table 2 results exhibited MIC against *E. coli* was determined at low concentration of LC 62.5 µg/mL and high concentration of 1000 µg/mL. The average percentage inhibition at these concentrations was 6.33281% and 86.0496%, respectively. As shown in the Figure 2, LC concentration of 62.5 µg/mL exhibited no significant inhibition. However, a significant increase in inhibition was observed with increasing LC concentrations, reaching a maximum of 86.0496% at 1000 µg/mL.

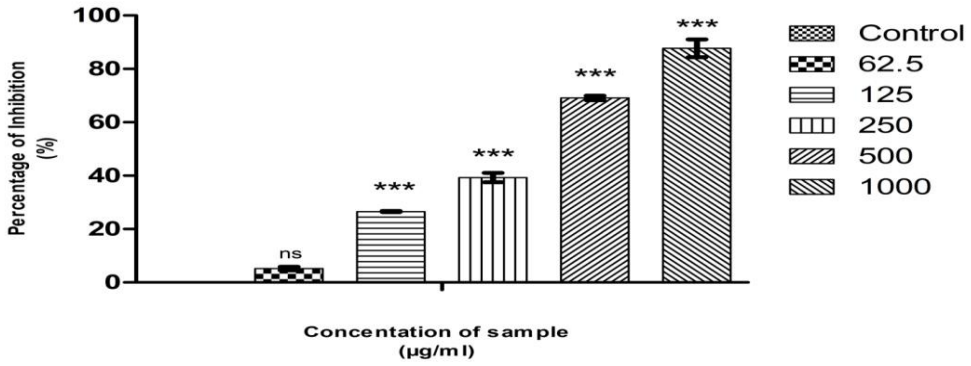

Figure 3: Graphical representation depicting the MIC of sample against *Pseudomonas aeruginosa*

Table 3 results revealed the MIC against *Pseudomonas aeruginosa* was determined at low concentration of LC 62.5 µg/mL and high concentration of 1000 µg/mL. The average percentage inhibition at these concentrations was 5.22055% and 87.7016%, respectively. As shown in the Figure 3, LC concentration of 62.5 µg/mL exhibited no significant inhibition. However, a significant increase in inhibition was observed with increasing LC concentrations, reaching a maximum of 87.7016% at 1000 µg/mL.

Table 3: Determination of Minimal Inhibitory Concentration against *Pseudomonas aeruginosa*

	% inhibition 1	*% inhibition 2*	*% inhibition 3*	*Average*	*Std*	*Std error*
Control	*0*	*0*	*0*	*0*	*0*	*0*
62.5 µg/mL	6.48664	4.99156	4.18344	5.22055	1.16855	0.38952
125 µg/mL	26.7121	26.167	26.8401	26.5731	0.35744	0.11915

250 µg/mL	41.4254	35.7987	40.6987	39.3076	3.06046	1.02015
500 µg/mL	67.6225	69.3898	70.4715	69.1613	1.4382	0.4794
1000 µg/mL	84.9944	83.7458	94.3646	87.7016	5.80399	1.93466

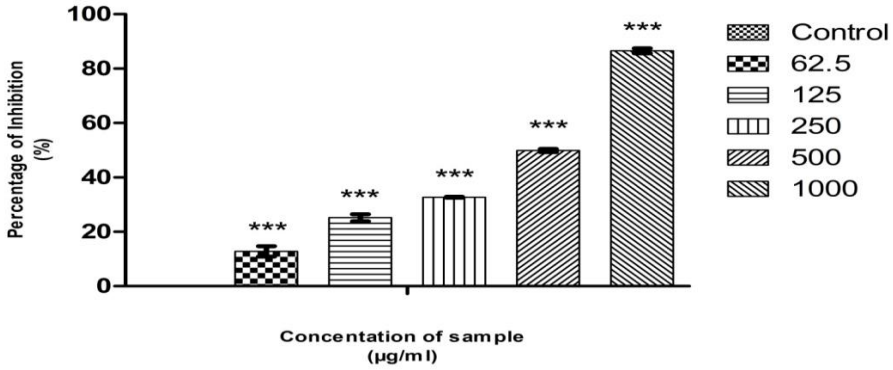

Figure 4: Graphical representation depicting the MIC of sample against *Staphylococcus aureus*

Table 4: Determination of minimal inhibitory concentration against *Staphylococcus aureus*

	% inhibition 1	*% inhibition 2*	*% inhibition 3*	*Average*	*Std*	*Std error*
Control	*0*	*0*	*0*	*0*	*0*	*0*
62.5 µg/mL	6.48664	9.97723	12.0237	16.3575	12.7862	3.25776
125 µg/mL	26.7121	25.088	22.9856	27.4969	25.1902	2.25739

250 µg/mL	41.4254	32.9331	32.3964	32.8781	32.7359	0.29527
500 µg/mL	67.6225	48.8512	50.0729	50.7134	49.8792	0.94611
1000 µg/mL	84.9944	85.5413	85.7901	88.3306	86.554	1.5436

Table 4: presents the Minimum Inhibitory Concentration (MIC) of LC against *Staphylococcus aureus*. The MIC was determined to be between 62.5 µg/mL (low concentration) and 1000 µg/mL (high concentration). The average percentage inhibition at these concentrations was 16.3575% and 88.3306%, respectively. As depicted in Figure 4**,** a significant increase in inhibition was observed with increasing LC concentrations, reaching a maximum of 88.3306% at 1000 µg/mL

Inhibitory Concentration 50 Value

Table 5: Inhibitory Concentration 50 Value of LC against difference pathogens

Pathogens	Inhibitory Concentration 50 Value
E coli	405.584 µg/mL
Pseudomonas aeruginosa	459.610 µg/mL
Staphylococcus aureus	515.575 µg/mL

Table No 5 presents the minimum inhibitory concentration 50 Values (MIC50) for the tested bacterial strains. The MIC50, representing the concentration at which 50% of bacterial growth is inhibited, was determined to be 405.584 µg/mL for *E. coli*, 459.61 µg/mL for *Pseudomonas aeruginosa*, and 515.575 µg/mL for *Staphylococcus aureus*. These values were calculated using ED50 PLUS V1.0 software.

Table 6: The minimum bactericidal concentration

Concentration	E coli		Pseudomonas aeruginosa		Staphylococcus aureus	
	No of colony counted	CFU/mL	No of colony counted	CFU/mL	No of colony counted	CFU/mL
Control (Organism alone)	303	$15.1*10^3$	609	$30.45*10^3$	234	$11.7*10^3$
LC 250 µg/mL	285	$14.2*10^3$	96	$4.8*10^3$	33	$1.65*10^3$
LC 1000 µg/mL	0	0	0	0	0	0

Table 6: Results demonstrating that a concentration of 1000 µg/mL of LC significantly inhibited the growth of all tested organisms. The minimum bactericidal concentration was 250 µg/mL, the remaining number of colonies *E.coli* 285 (14.2×10^3 CFU/mL), *Pseudomonas aeruginosa* 96 (4.8×10^3 CFU/mL), and *Staphylococcus aureus* 33 (1.65×10^3 CFU/mL).

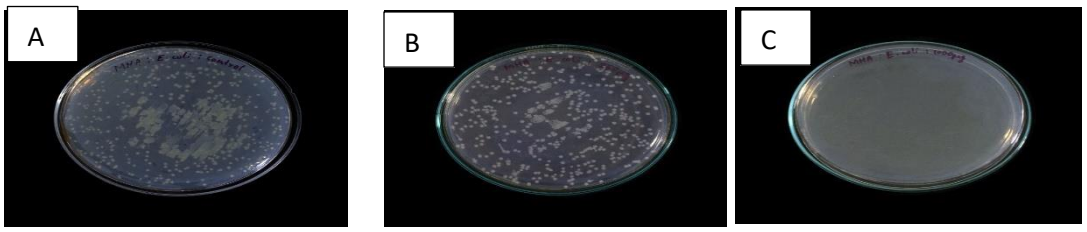

Figure 5: *E.coli* colonies A: control, B: LC 250 µg/mL C: LC 1000 µg/mL

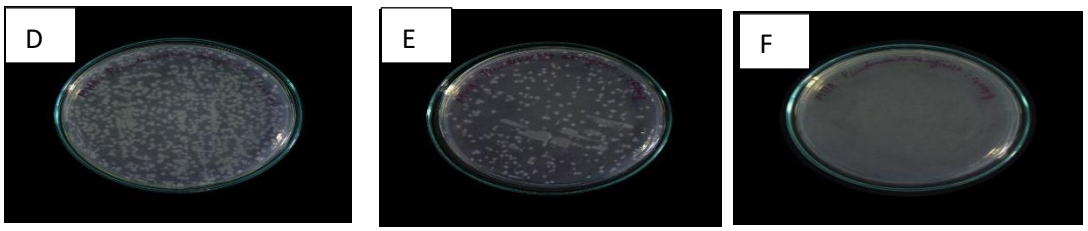

Figure 6: *Pseudomonas aeruginosa* colonies D: Control, E: LC 250 µg/mL, F:1000µg/mL

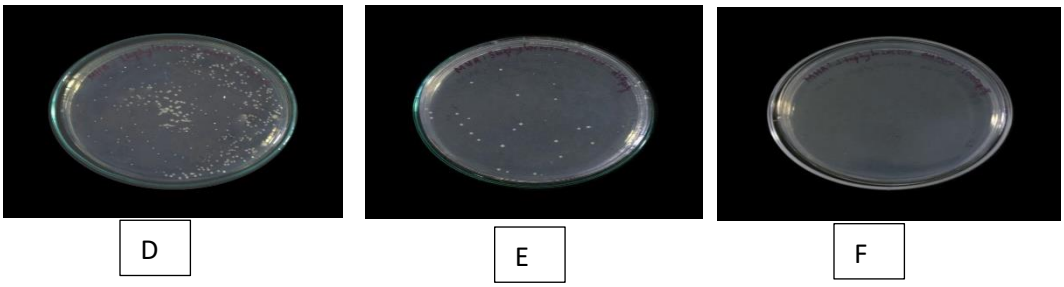

Figure 7: *Staphylococcus aureus* colonies G: Control, H: LC 250 µg/mL, I: 1000 µg/ mL

Figure 5, 6 and 7 provide a visual comparison of the distribution of microbial colonies in control and treated groups within the assay.

Conclusion

This study confirms that *Linga Chenduram* (LC) has antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. aureus*. While lower concentrations showed no zone of inhibition, 1000 µg/mL of LC consistently produced an 11mm zone of inhibition against all three pathogens. Further MIC experiments revealed a dose-dependent inhibition, with concentrations ranging from 62.5 µg/mL to 1000 µg/mL providing increasing effectiveness. At 1000 µg/mL, LC achieved significant inhibitions of 86.05% for *E. coli*, 87.70% for *P. aeruginosa*, and 88.33% for *S. aureus*. The Minimal Inhibitory Concentration 50 Values were 405.584 µg/mL (*E. coli*), 459.61 µg/mL (*P. aeruginosa*), and 515.575 µg/mL (*S. aureus*). Additionally, 1000 µg/mL of LC significantly reduced colony counts of all tested organisms, and the minimum bactericidal concentration (MBC) was 250 µg/mL. These findings suggest LC's potential as a natural antibacterial agent. Further *in-vivo* and clinical studies are recommended to fully assess its therapeutic efficacy.

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