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Antibacterial Activity and Phytochemical Analysis of Milagu Mezhugu – A Siddha Formulation

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Abstract: Infectious diseases remain a significant global health challenge, exacerbated by the growing problem of antibiotic resistance. Traditional medicine systems, such as Siddha, offer alternative therapeutic options with potential antimicrobial properties. Milagu Mezhugu, a Siddha formulation containing *Piper nigrum* (black pepper), is traditionally used for treating skin infections. This study aims to evaluate the antimicrobial activity and phytochemical profile of Milagu Mezhugu to assess its potential as an alternative to conventional antibiotics. The antimicrobial activity of Milagu Mezhugu was determined using the Resazurin Microtitre Assay to establish Minimum Inhibitory Concentrations (MIC) against various bacterial strains. Phytochemical analysis was conducted to identify the presence of bioactive compounds using standard chemical tests. Milagu Mezhugu demonstrated significant antibacterial activity, with the lowest MIC values observed against *Bacillus subtilis* (250 µg) and *Staphylococcus aureus* (500 µg). Higher MIC values were recorded for *Escherichia coli* and *Pseudomonas aeruginosa* (1000 µg). Phytochemical analysis revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids, and triterpenoids. Milagu Mezhugu shows broad-spectrum antimicrobial activity and a diverse array of phytochemicals, supporting its traditional use in treating infections. Further research is needed to explore its clinical applications and standardize its use in modern medicine.

Keywords: Milagu Mezhugu, *Piper nigrum*, Siddha formulation, Phytochemicals, Antibacterial, Antibiotics

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Introduction

The Siddha system of medicine, an ancient and comprehensive therapeutic tradition originating in India, is renowned for its integrative approach to health and disease management. This system is characterized by its emphasis on natural remedies, primarily herbal, which are used to restore and maintain equilibrium within the body's vital energies. Siddha medicine employs a

range of complex formulations designed to address various health conditions while minimizing adverse effects.

Infectious diseases pose a significant global health challenge, contributing substantially to the worldwide healthcare burden (Hemag *et al*., 2020). The increasing prevalence of antimicrobial resistance has exacerbated this issue, leading to heightened morbidity and mortality associated with bacterial infections. This growing crisis underscores the need for new therapeutic approaches, and medicinal plants, which have been utilized for their therapeutic properties since ancient times, present promising solutions due to their diverse bioactive compounds. (Iyswarya *et al*., 2023). Recent research highlights the potential of herbal and natural remedies in combating antimicrobial resistance.

The exploration and scientific validation of Siddha medicine's efficacy against contemporary infectious diseases offer an opportunity to integrate ancient practices with modern therapeutic needs. Siddha medicine employs complex formulations derived from a variety of medicinal plants. Among these, Milagu Mezhugu, a preparation based on *Piper nigrum* (black pepper), is traditionally used to treat skin conditions, including fungal infections (Deva asirvatham samuvel). *Piper nigrum*, a member of the Piperaceae family, contains bioactive compounds such as piperine and piperidine, which are known for their antimicrobial and anti-inflammatory properties (Meghwal and Goswami, 2013).

This study aims to evaluate the antimicrobial activity and phytochemical composition of Milagu Mezhugu, providing insights into its therapeutic potential and supporting its traditional use in managing infections.

Materials and Methods

Preparation of Milagu Mezhugu:

The preparation of Milagu Mezhugu was carried out at the Gunapadam laboratory, Sri Sairam Siddha Medical College and Research Centre following the traditional method with proper guidance.

Ingredients:

Milagu (*Piper nigrum*) – 50 g and Cow's butter – 25 g.

Procedure:

Milagu seeds were finely ground to a powder using a stone mortar and pestle. The cow's butter was added to the Milagu powder, and the mixture was thoroughly ground to form a homogeneous paste. The prepared paste was stored in a clean glass container, protected from contamination and stored at a controlled temperature to maintain its efficacy.

Antibacterial Activity:

The antibacterial activity of Milagu Mezhugu was assessed using the Resazurin Microtitre Assay, which determines the Minimum Inhibitory Concentration (MIC) of the formulation as reported by Sarker *et al*. (2007).

For this assay, a sterile microtiter plate with 96 wells was employed. The first well was spiked with 1000 µg of the sample solution dissolved in DMSO. Serial dilutions of the samples were prepared in the serials by transferring the solution to the next wells to obtain different concentrations of the sample. Each well was then inoculated with bacterial suspension containing the test organisms and the plate incubated at 37°C for 24-48 h. The last step comprised the addition of 100 µl of resazurin solution to all the wells. The transition of the colour from pink or complete colourless to blue indicated bacterial growth. MIC was identified as the minimum inhibition concentration of Milagu Mezhugu to which no colour change occurred indicating no bacterial growth (Fig. 1). Chloramphenicol (10 µg) was used as positive control to assess the efficiency of the bacterial strains.

Phytochemical Analysis (*Brain et al., 1975*):

Phytochemical screening was performed to identify and confirm the presence of various

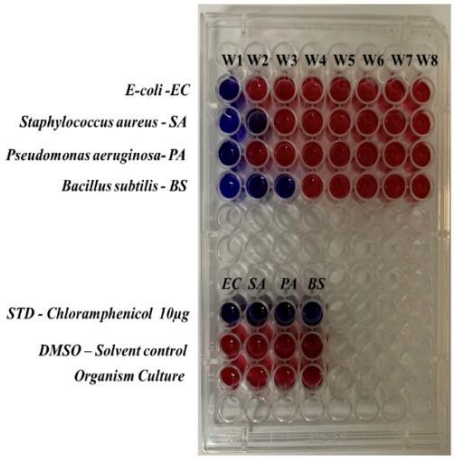

Fig. 1: 96 well plate - Antimicrobial Spectrum Image.

bioactive compounds in Milagu Mezhugu. The following tests were conducted:

Alkaloids: Mayer's Test was used, where a dull white precipitate indicated the presence of alkaloids.

Coumarins: Sodium hydroxide was added to the sample, and the formation of a yellow colour confirmed the presence of coumarins.

Saponins: A vigorous shake of the sample with water led to the formation of a frothy lather, indicating saponins.

Tannins: Ferric chloride was added to the sample, and a dark blue or greenish-black colour indicated tannins.

Glycosides: Borntrager's Test involved hydrolysing the sample with hydrochloric acid, followed by chloroform extraction and ammonia treatment. A pink colour indicated the presence of glycosides.

Flavonoids: An alkaline reagent test was performed by adding sodium hydroxide to the sample. The appearance of a deep yellow colour that faded with dilute hydrochloric acid confirmed the presence of flavonoids.

Phenols: Lead acetate was added to the sample, and a bulky white precipitate indicated phenolic compounds.

Steroids: A test with chloroform and concentrated sulfuric acid showed a red coloration in the chloroform layer and yellow-green fluorescence in the sulfuric acid layer, indicating steroids.

Triterpenoids: Liebermann–Burchard test was performed by adding acetic anhydride and sulfuric acid to the chloroform solution. The appearance of a red ring confirmed the presence of triterpenoids.

Cyanins (*Anthocyanin*): Sodium hydroxide was added to the sample, and a bluish-green colour indicated anthocyanins.

Carbohydrates: Benedict's test involved heating the sample with Benedict's reagent. A characteristic-coloured precipitate indicated carbohydrates.

Proteins: Biuret test was conducted by adding copper sulphate and sodium hydroxide to the sample. The formation of a violet-purple colour indicated proteins.

Results and Discussion

Antibacterial Activity:

The antibacterial activity of Milagu Mezhugu was evaluated against several bacterial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The results are summarized in Table 1.

The MIC values revealed that Milagu Mezhugu is most effective against *Bacillus subtilis* (MIC 250 µg), showing significant antimicrobial activity. It also exhibited notable activity against

Table 1: Growth Inhibition Chart for Milagu Mezhugu and Standard Drug

S. No.	Name of the Organism	MIC Value (µg)
1	*E. coli*	1000
2	*Staphylococcus aureus*	500
3	*Pseudomonas aeruginosa*	1000
4	*Bacillus subtilis*	250

Table 2: Phytochemical analysis of Milagu Mezhugu

Compound	Test Results
Alkaloids	Present (Mayer's test)
Coumarins	Present (Sodium hydroxide)
Saponins	Present (Froth test)
Tannins	Present (Ferric chloride)
Glycosides	Present (Borntrager's test)
Flavonoids	Present (Alkaline reagent)
Phenols	Present (Lead acetate)
Steroids	Present (Liebermann–Burchard test)
Triterpenoids	Present (Liebermann–Burchard test)
Cyanins (Anthocyanin)	Present (Sodium hydroxide)
Carbohydrates	Present (Benedict's test)
Proteins	Present (Biuret test)

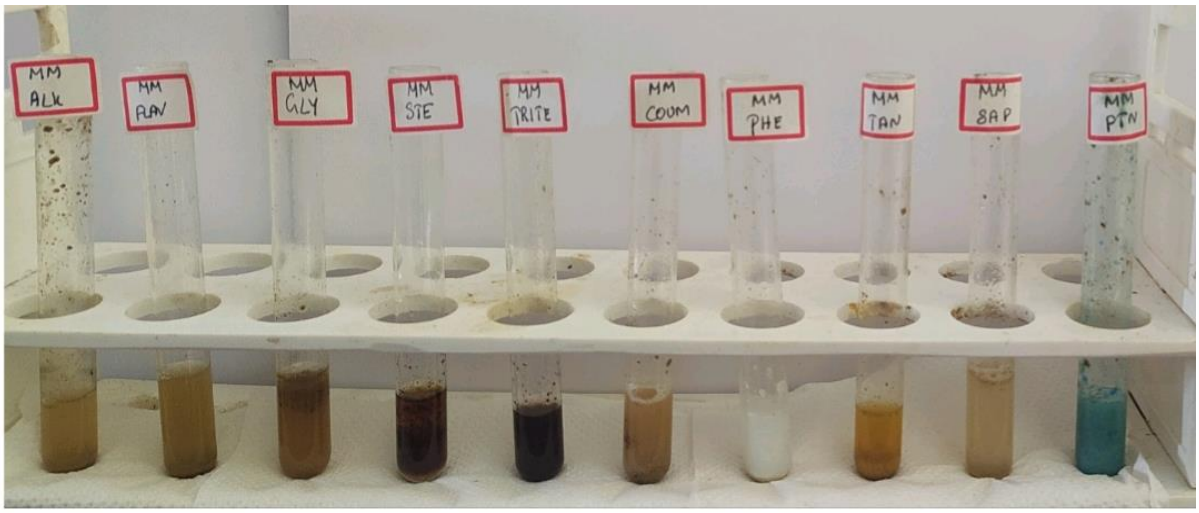

Fig. 2: Qualitative phytochemical analysis of Milagu Mezhugu.

Staphylococcus aureus (MIC 500 µg). However, *Escherichia coli* and *Pseudomonas aeruginosa* demonstrated higher MIC values (1000 µg), indicating less sensitivity (Table 1).

Phytochemical Analysis:

The phytochemical analysis identified several bioactive compounds in MilaguMezhugu, as detailed in Table 2 and Figure 2.

The phytochemical analysis of Milagu Mezhugu identified the presence of alkaloids, coumarins, saponins, tannins, glycosides, flavonoids, and phenols. Steroids, triterpenoids, cyanins, carbohydrates, and proteins were also

detected. These diverse compounds suggest a potentially rich therapeutic profile.

The study of Milagu Mezhugu, a traditional Siddha formulation incorporating *Piper nigrum* (black pepper), reveals significant findings regarding its antimicrobial efficacy and phytochemical composition. The results align well with its traditional uses and offer insights into the mechanisms underlying its effectiveness.

Antimicrobial Activity:

The Minimum Inhibitory Concentration (MIC) assay results illustrate that Milagu Mezhugu exhibits notable antimicrobial activity, particularly against *Bacillus subtilis* and *Staphylococcus aureus*. The MIC values of 250 µg for *Bacillus subtilis* and 500 µg for *Staphylococcus aureus* indicate a potent effect on these pathogens.

Bacillus subtilis: The low MIC value of 250 µg highlights MilaguMezhugu's strong efficacy against *Bacillus subtilis*. This aligns with the antimicrobial potential attributed to *Piper nigrum*. Studies have shown that components such as monoterpenes and sesquiterpenes, found in black pepper, exhibit significant antimicrobial properties (Ferrareze *et al*., 2007). These constituents may be responsible for the observed activity against *Bacillus subtilis* in the present study.

Staphylococcus aureus: The MIC value of 500 µg suggests substantial activity against *Staphylococcus aureus*, a common pathogen associated with serious infections. This finding is consistent with the antimicrobial activity reported for black pepper extracts, where piperine, a major component, has been shown to enhance bacterial cell membrane permeability, thereby facilitating the action of antimicrobial agents (Haq *et al*., 2021).

Escherichia coli and *Pseudomonas aeruginosa*: The higher MIC values (1000 µg) for *Escherichia coli* and *Pseudomonas aeruginosa* indicate reduced effectiveness against these Gram-negative bacteria compared to *Bacillus subtilis* and *Staphylococcus aureus*. This reduced efficacy may be due to the advanced resistance mechanisms of these bacteria, such as efflux pumps and protective outer membranes that can limit the penetration of antimicrobial agents (Mattioli *et al*., 2020; Enaru *et al*., 2021).

Phytochemical Profile:

The phytochemical analysis of Milagu Mezhugu revealed a diverse range of bioactive compounds, including alkaloids, saponins, tannins, flavonoids, and triterpenoids, which contribute to its antimicrobial properties:

Alkaloids: These compounds are known for their broad-spectrum antimicrobial activity. Their presence in Milagu Mezhugu is likely a major factor in its effectiveness, as they can disrupt various bacterial functions and contribute to overall antimicrobial activity (Othman *et al*., 2019; Jafaar *et al*., 2021).

Saponins: Saponins have antimicrobial properties due to their ability to disrupt microbial cell membranes. Their presence in Milagu Mezhugu could enhance its effectiveness against a range of pathogens by causing leakage of cellular contents.

Tannins: Tannins are known to bind and precipitate proteins, which can inhibit bacterial growth by affecting protein functions. Their presence in Milagu Mezhugu likely contributes to its antimicrobial activity (Enaru *et al*., 2021).

Flavonoids and Triterpenoids: Flavonoids can modulate microbial cell membranes and inhibit essential bacterial enzymes. Triterpenoids are recognized for their antimicrobial and anti-inflammatory properties. Both types of compounds support the antimicrobial potential of MilaguMezhugu (Mattioli *et al*., 2020).

Limitations and Future Research:

While this study provides valuable insights into the antimicrobial potential and phytochemical composition of Milagu Mezhugu, there are limitations. The study focused on *in vitro* evaluations; hence, the clinical efficacy and safety of Milagu Mezhugu require further investigation. Future research should include clinical trials to

assess its therapeutic potential in humans and identify any possible side effects. Additionally, the optimization of the formulation and the standardization of its preparation will be essential for its integration into modern medical practice.

Conclusion

Milagu Mezhugu exhibits significant antimicrobial activity and contains a broad spectrum of bioactive phytochemicals. The findings align with its traditional uses and highlight its potential as a therapeutic agent against bacterial infections. Further research is needed to validate its clinical efficacy and explore its role in addressing the global challenge of antimicrobial resistance.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Department of Gunapadam, National Institute of Siddha, Ministry of Ayush, Govt. of India, Chennai, Tamil Nadu, India.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare no conflicts of interest.

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