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In Vitro Antitubercular Activity of Pippalyadi Avaleha

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ABSTRACT

In Ayurvedic medicine, Pippalyadi Avaleha is a traditional polyherbal formulation that has been studied for its potential anti-tubercular properties. The primary component, Piper longum (Pippali), is known for its immunomodulatory, anti-inflammatory, and bronchodilator effects, which are beneficial in managing respiratory conditions like TB. Pippalyadi Avaleha is believed to enhance pulmonary function, modulate the immune response, and provide symptomatic relief in TB patients. Integrating Pippalyadi Avaleha into a multidisciplinary treatment regimen may offer complementary benefits, particularly in cases of multidrug-resistant TB. The pharmacological actions of this Ayurvedic formulation, including its ability to enhance host immunity and reduce pulmonary inflammation, support its use as an adjunct therapy in TB management. This approach aligns with the need for novel treatments that can address drug-resistant TB strains while supporting overall patient health. Tuberculosis (TB), caused by Mycobacterium tuberculosis, is a serious infectious disease that primarily targets the lungs but can disseminate to other organs. The increasing incidence of multidrug-resistant TB (MDR-TB) poses a significant challenge to conventional treatment, necessitating the exploration of alternative therapeutic approaches.

Keywords: Tuberculosis, Pippalyadi Avaleha, Ayurvedic Medicine, Antitubercular Activity, Multidrug-Resistant TB, Piper Longum.

INTRODUCTION

Tuberculosis (TB) continues to be a pressing global health challenge, particularly with the growing presence of multidrug-resistant and extensively drug-resistant Mycobacterium tuberculosis strains. The emergence of these resistant strains has intensified the need for alternative treatment options. Ayurveda, the ancient system of Indian medicine, offers a vast array of formulations with potential therapeutic properties. One such formulation, Pippalyadi Avaleha, has recently been highlighted for its possible effectiveness against tuberculosis.

Aim: To evaluate the antitubercular activity of Pippalyadi Avaleha.

Objective: To Determine the efficacy of Pippalyadi Avaleha in inhibiting the growth of Mycobacterium tuberculosis in vitro studies.

Methods and Material:
Place of Work
Preparation of Pippalyadi Avaleha was carried out in Pharmacy of Department of Rasashastra and Bhaishajya Kalpana, School of Ayurved, D.Y. Patil Deemed to Be University, Nerul, Navi Mumbai.

Reference of the Pippalyadi Avaleha for study purpose is taken from Gada Nigraha.

Pippalyadi Avaleha

कृष्णाचूर्णक्षिपेत्प्रस्थंसिताप्रस्थद्वयंतथा।प्रस्थार्धगोघृतंचैवकुडवंमाक्षिकंतथा॥ 300॥
दुग्धाढकेनसंयुक्तंयथोक्तंविपचेद्भिषक्।चातुर्जातपलंचैकंचूर्णमेतद्विनिक्षिपेत्॥301॥
प्रत्यूषेभक्षयेन्नित्यंततःकार्यंसमाचरेत्।हन्त्यष्टादशकुष्ठानिक्षयमेकादशात्मकम्॥302॥
पञ्चकासाँस्तथाश्वासान्पाण्डुंप्लीहमपस्मृतिम्।मूत्रकृच्छतथारक्तंशुक्रदोषंतथाजराम्॥303॥
धातुक्षयंचमन्दाग्निंव्याधिंपरमदुस्तरम्।सर्वांस्तान्नशयत्याशुतमःसूर्योदयोयथा॥304॥
सुभगोदर्शनीयश्चसगच्छेत्प्रमदाशतम्।रसायनमिदंश्रेष्ठमश्विभ्यांपरिकीर्तितम्॥ 305॥
Gada Nigraha, Prayoga Khanda, Lehadhikar (300-305)

Procurement and Authentication
Raw materials were procured from authentic suppliers, Identification and selection was done and procured.

Table No. 1 - Ingredients mentioned in above verses with quantity

Sr. No.	Ingredients	Quantity
1	Pippali	768 g
2	Go Dugdha	3072 ml
3	Go Ghruta	384 g
4	Sita	1536 g
5	Twak	12 g
6	Ela	12 g
7	Patra	12 g
8	Nagakesar	12 g
9	Madhu	192 g

SAMPLE DESCRIPTION		: Samples labeled as –
Sr. No.	Description	
1.	Pippalyadi Avaleha	

Preparation Of Pippalyadi Avaleha by Standard Procedure

Preparation Start Date
05/03/2024.

Finished Product Date
06/03/2024.

Experimental Study of Pippalyadi Avaleha
In-vitro anti-tubercular activity of Pippalyadi Avaleha with minimum inhibitory concentration (MIC). A variety of strains like H37Rv, Sensitive, and atypical strain are used for detection of anti-Tb activity.

Place of Work
Anti-tubercular study was conducted at Department of Microbiology, K. J. Somaiya Medical College & Research Institute, Mumbai.

1. **Study Title**
   Assessment of in vitro anti-tubercular activity of Pippalyadi Avaleha.

1. **Name of Test**
   Minimum inhibitory concentration (MIC)

2. **Test Method**
   NCCLS document M31-A2; 2015

3. **Test Materials**
   Sample of Pippalyadi Avaleha

4. **Study Design**
   In this in-vitro study, the Minimum Inhibitory Concentration (MIC) value of Pippalyadi Avaleha was determined on standard strain (H37Rv), Sensitive, and atypical strains of Mycobacterium Tuberculosis.

5. **Test Organism**
   a. Atypical Mycobacterium (Obtained from Patients, K. J. Somaiya Medical College)
   b. Mycobacterium Tuberculosis (Obtained from Patients, K. J. Somaiya Medical College)
   c. Standard Strain H37Rv (Obtained from J. J. Hospital, Traceable NTI, Bangalore)

6. **Purpose**
   The MIC (minimum inhibitory concentration) is the lowest drug concentration that inhibits the growth of a particular bacterial isolate. MIC is an in vitro measurement of bacterial susceptibility. The lower the MIC value, the more susceptible the isolate is to that drug. MICs are determined using serial two-fold dilutions of drug to which a standardized inoculum is added and incubated for a specified time. For provided samples and Mycobacteria as test organism, MIC by Agar dilution method is used. In this, serial two/ multi fold dilution of the products are made in Middle Brook agar. The test organisms are then added to the dilutions of the products, incubated, and scored for growth.

7. **Experimental Conditions**
   Media : Middle Brook agar with supplement
   Drug : Pippalyadi Avaleha
   Incubation : 37°C for 2-6 weeks

8. **Procedure**

- The given product Pippalyadi Avaleha were water insoluble, so they were solubilized in 10% DMSO.
- The given product was diluted from 10% to 0.321% by serial 2-fold dilution using Middle Brook growth medium.
- Test organisms viz. Atypical Mycobacteria, Mycobacterium Tuberculosis was diluted to culture density 10^8 CFU/ml. They were further diluted to 10^6 CFU/ml. This was used as inoculum.
- In graded test product tubes, culture of Mycobacterium was spread throughout the slant portion. Adequate Positive and Negative controls were put up. Tubes were incubated at 37°C for 2-6 weeks.
- Tubes showing growth of test organism was reported as growth or No growth when there was absence of growth.

OBSERVATION AND RESULTS

Strain H37RV

Mycobacterium tuberculosis standard strain

ATYPICLE MYCOBACTERIUM

Results:

Conc. of Test Product	Atypical Mycobacterium	Mycobacterium tuberculosis	Mycobacterium tuberculosis H37RV
10% (100 mg/ml)	No Growth	No Growth	No Growth
5% (50 mg/ml)	No Growth	No Growth	No Growth
2.5% (25 mg/ml)	No Growth	No Growth	No Growth
1.25% (12.5 mg/ml)	Growth	No Growth	Growth
0.625% (6.25 mg/ml)	Growth	Growth	Growth
0.312% (3.12 mg/ml)	Growth	Growth	Growth
MIC	2.5% (25 mg/ml)	1.25% (12.5 mg/ml)	2.5% (25 mg/ml)

RESULTS

There was no growth seen at the concentration of 25mg/ml of Atypical Mycobacteria and there was no growth seen at the concentration of 12.5mg/ml of Mycobacterium Tuberculosis drug sensitive strain, no growth seen at the concentration of 25mg/ml on standard strain H37Rv. So, the minimum inhibitory concentration or the concentration at which the growth of the mycobacterium Tuberculosis is inhibited is 25mg/ml.

DISCUSSION

The present work "In vitro anti-tubercular activity of Pippalyadi Avaleha" was executed with understanding and proper interpretation of various concepts of Ayurved, Standard Operating Procedures and Assays. Based on the study, observations and results, all points are discussed with proper reasoning.

Experimental Study-Anti-Tubercular Activity of Pippalyadi Avaleha
There are several in-vitro methods for screening anti-tubercular activity. Minimum Inhibitory Concentration is the most conventional method used in Preclinical Tuberculosis drug testing for new drug. A variety of strains like H37Rv, Sensitive, and atypical strain are used for detection of anti-Tb activity.

The MIC (minimum inhibitory concentration) is the lowest drug concentration that inhibits the growth of a particular bacterial isolate. MIC is not a measure of efficacy per se, but instead it is simply an in vitro measurement of bacterial susceptibility. The lower the MIC value, the more susceptible the isolate is to that drug.

MICs are determined using serial two-fold dilutions of drug to which is added a standardized inoculum that is incubated for a prescribed time. MIC test is conducted, according to strict procedural standards, including quality control, such as those in NCCLS document M31-A2.

For provided sample and Mycobacteria as test organism, MIC by Agar dilution method is used. In this, serial two-fold dilutions of the product are made in middle brook agar media. The test organisms are then added to the dilution of the product, incubated and scored for the growth.

3.12mg/ml, 6.25mg/ml, 12.5mg/ml, 25mg/ml, 50mg/ml and 100mg/ml concentration of Pippalyadi Avaleha was selected for the Minimum Inhibitory Concentration. Positive controls were put up.

There was no growth seen at the concentration of 25mg/ml of Atypical Mycobacteria and there was no growth seen at the concentration of 12.5mg/ml of Mycobacterium drug sensitive strain, no growth seen at the concentration of 25mg/ml on standard strain H37Rv. So, the minimum inhibitory concentration or the concentration at which the growth of the mycobacterium Tuberculosis is inhibited is 25mg/ml.

This shows positive activity of Pippalyadi Avaleha. It has anti-tubercular activity on standard strain as well as sensitive strains.

Probable Mode of Action

- Pippalyadi Avaleha contains ingredients Pippali which has active component viz. Piperine. In the previous research it has been proven that Piperine act as anti-tubercular, anti-mycobacterial, anti-microbial, and help as bioavailability enhancer. Pippalyadi Avaleha has Tikta and Madhur Rasatmak Guna which makes formulation in palatable form. Use of Madhur Dravya is of great importance which has a "Shad Indriya Prasadak Guna" & it nourishes all dhatus. Pippalyadi Avaleha formulation can be one of the therapeutic strategies in Ayurvedic medicines is to enhance the bioavailability of anti-tubercular drugs, and Madhur guna to help in increasing strength of body.
- Pippalyadi Avaleha contains Pippali, which is known for their immunomodulatory properties. It may help to enhance the body's immune response, potentially aiding in the clearance of Mycobacterium Tuberculosis, the bacterium that causes TB.
- **Anti-inflammatory Effects**: Pippali, have demonstrated anti-inflammatory properties. Chronic inflammation plays a role in the pathogenesis of TB, and reducing inflammation may help mitigate tissue damage and improve clinical outcomes in Tuberculosis patients.
- **Antioxidant Activity**: Pippali, possess antioxidant properties. Oxidative stress is implicated in the progression of Tuberculosis and the development of complications. Antioxidants may help neutralize free radicals and reduce oxidative damage to tissues. Pippalyadi Avaleha may contribute to better outcomes in Tuberculosis patients by enhancing vitality and resilience, by supporting the body's natural healing mechanism.

CONCLUSION

The study's findings suggest that Pippalyadi Avaleha could serve as a complementary treatment option, potentially enhancing the efficacy of existing TB therapies and offering hope for patients with drug-resistant forms of the disease. However, to fully realize its potential, comprehensive clinical trials are necessary to assess its safety and effectiveness in human subjects. By bridging the gap between traditional Ayurvedic practices and modern scientific research, Pippalyadi Avaleha could pave the way for innovative approaches to tuberculosis treatment. The integration of Ayurvedic formulations like Pippalyadi Avaleha into conventional medicine could not only provide additional therapeutic options but also promote a more holistic approach to patient care.

Furthermore, exploring the pharmacological properties of the ingredients in Pippalyadi Avaleha could yield valuable insights into their mechanisms of action against Mycobacterium tuberculosis. This could lead to the identification of active compounds that may be effective in combating drug-resistant strains of the bacteria.

In addition to clinical trials, collaboration between Ayurvedic practitioners and modern medical researchers could facilitate a better understanding of how traditional remedies can complement existing treatments. This interdisciplinary approach could ultimately enhance patient outcomes and contribute to the global fight against tuberculosis.
As research progresses, it will be essential to ensure that any complementary treatments are rigorously evaluated for safety and efficacy, adhering to the standards of modern medical practice. By doing so, we can honor the wisdom of traditional medicine while ensuring that patients receive the best possible care based on scientific evidence.

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