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Antimicrobial Study with MIC Test to Determine the Lowest Concentration of a Shwasahara (Anti Asthmatic) Ayurvedic Formulation for Inhibiting the Visible Growth of a Gram Positive Bacteria (*S. aureus*) & a Gram Negative Bacteria (*E. coli*)

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Abstract

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties. *Staphylococcus aureus* (*S. aureus*) is one of the most common causative agent of multiple human infections including, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, urinary tract infections etc[3] and *Escherichia coli* (*E. coli*) is the most common pathogen leading to uncomplicated cystitis, and also results in other extra intestinal illnesses, including pneumonia, bacteremia, and abdominal infections such as spontaneous bacterial peritonitis[4]. Therefore this study is focused on, to determine the lowest concentration of a Shwasahara (Anti Asthmatic) ayurvedic formulation composed of Bharangi (*Clerodendrum indicum* Linn.), Sunthi (*Zingiber officinale* Rosc.), and Pippali (*Piper longum* Linn) for inhibiting the visible growth of *S. aureus* and *E. coli*.

Keywords: Antimicrobial study, MIC (Minimum Inhibitory Concentration) Test, *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), Bharangi + Sunthi + Pippali

1. Introduction

Emergence and dissemination of multidrug resistant (MDR) strain in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria [5, 6]. According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs [7]. Because of the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to find out the lowest concentration of a shwasahara (Anti Asthmatic) ayurvedic formulation for inhibiting the visible growth of *S. aureus* and *E. coli*. The formulation consists of Bharangi (*Clerodendrum indicum* Linn.), Sunthi (*Zingiber officinale* Rosc.), and Pippali (*Piper longum* Linn), is described in 'Yogaratnakar' for the treatment of shwas disorders [8]. The constituent herbs of this formulation are reported for having anti-microbial activity such as Bharangi (*Clerodendrum indicum* Linn.)-the methanolic extract and its different fractions of leaves of *Clerodendrum indicum* possess significant anti-nociceptive, antimicrobial and antidiarrheal activities [9]. Sunthi (*Zingiber officinale* Rosc.)-Study conducted by Auta *et al*. (2011) the antimicrobial property of various extract of *Zingiber officinale* was investigated against *Escherichia coli* and Pseudomonas aeruginosa using the Agar and tube diffusion method. The result obtained revealed that ethanolic extract of ginger gave the widest zone of inhibition against one out of the two test organisms at the concentration of 20 mg/ml. However, Pseudomonas aeruginosa was more sensitive to the extract [10]. Pippali (*Piper longum* Linn)-the extracts of fruits of *Piper longum* has the anti-oxidative, anti-microbial and anti-apoptotic activity due to the possession of pharmacologically and medicinally significant phytochemicals suggesting its therapeutic usefulness to cure respiratory afflictions caused either by microbial activity or physiological stress caused by the overproduction of free radicals which may lead to the cancer [11].

2. Materials and Methods

There were Three Main Reagents to Run this Study: The media, antimicrobial agents or plants extract and the microbes being tested. Depending on the pathogens and plants extract being tested, the media was adjusted. The antimicrobial concentration was adjusted into the correct concentration by

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mixing stock antimicrobial with media. The adjusted antimicrobial was serially diluted into multiple tubes to obtain a gradient. The dilution rate adjusted depending on the breakpoint and needs. The microbe or the inoculating agent must be at the correct concentration. So, this was adjusted by incubation time and dilution. For verification, the positive control (tetracycline hydrochloride) is placed in a hundred fold dilution to count colony forming units. The microbes inoculate the tubes and were incubated for 16-20 hours. The MIC is generally determined by turbidity.

i). Preparation of Simple media or basal media is consists of peptone, meat extract, yeast extract, sodium chloride and water. Which is known as nutrient broth. Ingredients is showing below-
Distilled water-1L
Beef extract-1g
Yeast extract-2g
Peptone-5g
Sodium chloride (NaCl)-5g

Above mentioned ingredients were weighing properly and taken in 1000ml conical flask. Then conical flask is placed on stir plate for mixing the nutrient broth. Lastly, pH of the nutrient broth was tested, the pH = 7.

i). Preparation of Inoculum Total 16 numbers of test tubes taken which were cleaned and moisture free in hot air oven. 6ml of nutrient media was poured individually and the opening of test tubes were sealed by cotton plugs and securely wrapped by papers and rubber bands. These were placed for sterilization in auto-clave machine.

ii). Microbes were selected one gram +ve *Staphylococcus aureus* and another is gram -ve *Escherichia coli* for this study.

iii). After sterilization of test-tubes were labeled for desiring tests. Microbes were subjected accordingly and kept for 48hrs in incubator, 120RPM at 37°C. All methods were performed in laboratory hygienic protocols.

iv). Preparation of antimicrobial agent or plant extract: Stock solution of research drug was made. Two number of 15ml disposable plastic centrifuge tube were taken and here 2gm of extract was dissolved in 10ml distilled water.

v). After 24hrs of incubation test tubes were taken out and plants extract of formulation was subjected in 200 mg/mL, 300 mg/mL, 400mg/mL to observe MIC of formulation on *E. coli*. Further study has done with *E. coli* and *S. aureus* in 400mg/ml, 500mg/ml, 600mg/ml concentration of formulation to justify the previous study on low doses. Again those were incubated for 24 hrs. In the both of the above studies, tetracycline hydrochloride (0.1mg/ml) and nutrient broth with bacteria were used as a positive control and negative control respectively.

3. Observations and Results

The understanding of the mechanism of antimicrobial action of medicinal plants extracts is the first step in the optimal utilization of these extracts as natural antimicrobial agents to extend the shelf-life and maintain the drug quality. In the current investigation carried out, a screening of aqueous extracts of formulation (Bharangi + Sunthi + Pippali) against gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. Coli*) was done to detect the minimum concentration of the formulation to show antimicrobial activity against the bacteria.

Table 1: Showing result after 24hrs inoculating tubes (High-Low turbidity)

Research drug (RD4)	Microbes	200 mg/mL	300 mg/mL	400 mg/mL
Formulation (Bharangi + Sunthi + Pippali)	*E. coli*	+++++	++++	+++

Note: +++++sign indicating high turbidity and +++ sign denotes low turbidity in comparison to positive control and negative control

Fig 1: Representing the MIC study of research drug (RD4) on E. coli in the concentration of 200mg/ml, 300mg/ml, 400mg/ml where test tube labeled as T.C. is the test tube with tetracycline hydrochloride (0.1mg/ml)[Positive control] & test tube labeled as *E. coli* was negative control

Impression: Anti-microbial study after 24 hour observation, indicates the MIC of research drug formulation found at the dose of 400mg/ml. To confirm the observation, further study has been done with 500mg/ml, 600mg/ml concentration of research drug formulation.

Table 2: Showing result after 24hrs inoculating tubes (High-Low turbidity)

Research drug (RD4)	Microbes	400 mg/mL	500 mg/mL	600 mg/mL
Formulation	*S. aureus*	+++	++	+
Formulation	*E. coli*	+++	++	+

Note: +++sign indicating moderate turbidity double folds increased and + sign denotes very low turbidity with standards.

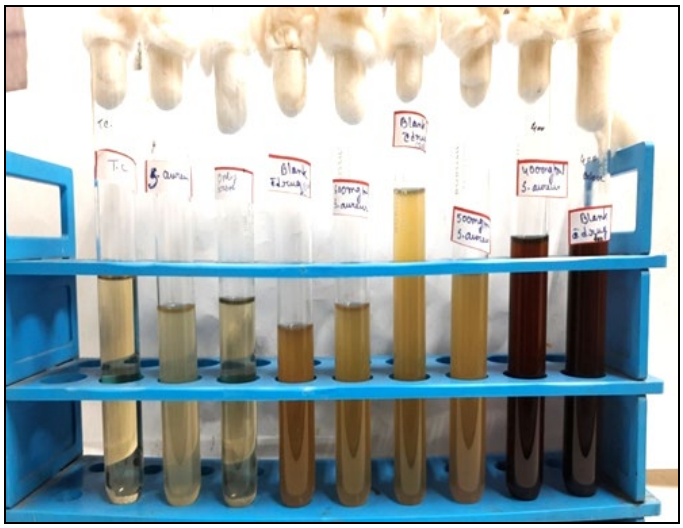

Fig 2: Representing the MIC study of research drug(RD4) on *S. aureus*, in the concentration of 400mg/ml, 500mg/ml, 600mg/ml where test tube labeled as T.C. is the test tube with tetracycline hydrochloride (0.1mg/ml)[Positive control] & test tube labeled as *S. aureus* was negative control

Fig 3: Representing the MIC study of research drug (RD4) on *E. Coli* in the concentration of 400mg/ml, 500mg/ml, 600mg/ml where test tube labelled as T.C. is the test tube with tetracycline hydrochloride (0.1mg/ml)[Positive control] & test tube labelled as *E. coli* was negative control

Impression: The antimicrobial study of the formulation was to find out the effect of this formulation on gram +ve bacteria *S. aureus* and on gram -ve bacteria *E. Coli* following MIC method. And the study showed, MIC started from the dose of 400mg/ml.

4. Discussion

The medicinal plants are god's gift to cure a number of diseases among the living organisms. In the current study, a screening of aqueous extracts of formulation (Bharangi + Sunthi + Pippali) against gram +ve bacteria (*Staphylococcus aureus*) and gram -ve bacteria (*E. coli*) was done in order to detect its minimum concentration for antimicrobial activity. The study showed, MIC started from the dose of 400mg/ml. Initially the study has been conducted with the dose of 200mg/ml, 300mg/ml and 400mg/ml with microbe *E. coli*, after 24 hr observation it was observed that MIC started from the dose 400mg/ml. To confirm the authenticity of the study further study has been done in the dose of 400mg/ml, 500mg/ml and 600mg/ml with microbes *E. coli* and *S. aureus* and here too, after 24 hr observation, MIC has been shown to start from 400mg/ml dose of the research drug formulation.

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