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Evaluation of antimicrobial activities of Ayush Siddha formulations in the management of childhood illness

D. S. Vaniswari*[1], R. Remya[*1] , K.Shyamala[2]

[*1]PG scholars, Department of Kuzhanthai Maruthuvam, Govt.Siddha.Medical.College, Palayamkottai.

[2]Lecturer, Department of Kuzhanthai Maruthuvam, Govt.Siddha.Medical.College , Palayamkottai, Tamilnadu, India.

*Corresponding Authors: **D.S.Vaniswari*, R.Remya***

[*]PG scholars ,Department of Kuzhanthai Maruthuvam, Govt.Siddha. Medical College, Palayamkottai, Tamilnadu, India. E-mail: *dsvani29@gmail.com*

Abstract

Respiratory tract infections by *Staphylococci aureus, Streptococci pneumoniae, Klebsiella pneumoniae* and *Escherichia coli* deserve special attention in pediatric age groups especially in India because of their frequency and because they are caused by strains resistant to various antibiotics. Furthermore ,the most dramatic increases are occurring in the pediatric age group. The aim of the present study is to screen *In-vitro* Anti-microbial activity of Siddha formulations against human pathogenic micro organism causing respiratory tract infections.

Keywords: Siddha polyherbal formulation, Anti microbial assay, Respiratory tract infections.

Introduction

Luxuries of modern world has brought many comforts in the life of human being, but it has also inevitable bought many health hazards owing to changed environmental conditions. In Industrialised nations, espite the progress made in the understanding of microbiology and their control, incidents of epidemics due to drug resistant microorganism and the emergence of hitherto unknown disease-causing microbes, pose enormous public health concerns. In order to halt the trend of increased emerging and resistant infectious disease,requires a multi-pronged approach that includes the development of new antimicrobial drugs.

Potentiality of Siddha formulation in Antimicrobial assay:

In Modern medicine, treatment of Respiratory tract infection should be given on the basis of sensitivity testing .Antimicrobial agents are used for the treatment. Since from Ancient times, Siddha medicine have provided a good source of antimicrobial agents. Siddha medicinehave forever been a catalyst for healing. Interest in a large number of traditional Siddha medicinal usage has increased today. It has been suggested that extracts from Siddha herbals used in allopathic medicine are potential sources of antiviral, antitumoral and anti microbialagents.

The selection of Siddha medicinal formulations for screening program has the potential of being more successful in initial steps than the screening of isolated pure compounds.

Materials and Methods

Method of preparation of Test samples:

Test samples used in this present study are Ayush Siddha formulations namely, Ellanthaiellai kudineer chooranam, Vaasaikudineer chooranam, Atti chooranam, Arathaai chooranam and Aatruthumattimelugu which are prepared as per Siddha Materia Medical procedures.

Preparation of extract of Test samples:

Aqueous Extraction:

Ingredients of test samples were purified ,air dried and then homogenised to fine powder and stored in airtight bottles as per Siddha Materia Medical procedures .For aqueous extraction,10 gm of air-dried powder was placed in distilled water and boiled for 6 hours. At intervals of 2 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected. After 6 hours, The supernatant was concentrated to make the final volume one-fourth of the original volume. Finally 10g of material was extracted in 25 ml of distilled water giving a concentration of 40mg/0.1ml.It was then autoclaved at 121 ^{o}C and 15 Ibs pressure and stored at 4^{o}C.

Disc Diffusion Method

Principle:

The placing of a filter paper disc (measured 6mm in diameter) containing known amount of an antimicrobial agent on the agar surface previously inoculated with bacterium to be tested will result in zone of inhibition of growth around the disc.

Culture Media used: Muller-Hinton Agar Media (M173)

Kirby-Bauer Disc Diffusion Method: It is a simple and reliable method applicable in routine clinical bacteriology

Requirements

Culture medium: Muller – Hinton agar (M173).It supports the growth of most of the organisms.

Test Micro organisms:

The microbial strains are identified strains and were obtained from the Malar Micro Diagnostic Center, Tamilnadu, India and maintained in the laboratory by periodic subculture. The pathogenic bacterial strains studied are *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus.*

Inoculum of test bacterium used in a suitable broth medium (eg. Peptone water)

For preparation of inoculum, pure culture of the test micro organisms are inoculated into a broth medium and incubated at 37 C for 2-4 hours.

Procedure

- A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 h to activate the strain. Muller Hinton Agar (M 173) was prepared for the study. The assay was performed using Agar disk diffusion for aqueous extract .The media and the test bacterial cultures were poured into Petri dishes(Hi-Media).
- The test strain (0.2 ml) was inoculated into the media (inoculum size 108 cells/ml) when the temperature reached 40-42 C. Care was taken to ensure proper homogenisation. The experiment was performed under strict aseptic conditions. For the Agar disk diffusion method ,the test compound (0.1ml) was introduced

onto the disk(0.7cm)(Hi-Media)and then allowed to dry. Thus the disk was completely saturated with the test compound.Then the disk was introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37C.

- Microbial growth was determined by measuring the diameter of the zone of inhibition. Distilled water was used as the control. The control activity was deducted from the test and the result obtained was plotted. The antibacterial activity of Siddha formulations extract of aqueous solvents against selected pathogenic bacterial strains were screened.
- A standard control Amikacin is also tested for comparison. The zone of inhibition was measured with the scale from the centre of disc to the clear zone in millimetre and the results were recorded.
- The plates are incubated at 37C for 16-18 hours and susceptibility is determined on the basis of zone of inhibition

Results

The results of In vitro anti-microbial assay indicates that aqueous extract of test samples showed more anti-bacterial activity against human pathogenic micro organisms responsible for nosocomial infections , with the standard control Amikacin for comparison. Results were expressed in table and Figure:

Anti bacterial activity of Test samples:

Test sample Siddha formulations	Test Micro organisms	Zone of Inhibition size of Siddha formulations	Zone of Inhibition size of control(Amikacin)
Ellanthaiellai kudineer chooranam	*Streptococcus pneumoniae* *Staphylococcus aureus*	20 mm 19 mm	20 mm 20 mm
Vaasaikudineer chooranam	*Streptococcus pneumoniae* *Escherichia coli*	20 mm 19 mm	22mm 22mm
Attichooranam	*Escherichia coli*	20 mm	22mm
Arathaai chooranam	*Klebsiella pneumoniae*	15 mm	16 mm
Aatruthumattimelugu	*Escherichia coli* *Staphylococcus aureus*	11mm 19mm	12mm 20mm

Discussion

Aqueous extract of Test samples were subjected to anti-microbial studies. This study revealed that the above test samples of siddha formulations can be prescribed to Respiratory tract infections due to *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and Escherichia coli which deserve special attention in pediatric age groups.

Conclusion

The efficacy of Siddha formulation against bacterial strains included in this study which will encourage the young researchers to carryout further research. Siddha medicines showed maximum antibacterial activity and so these medicines may serve as leads for the development of new antimicrobials against drug resistant respiratory tract pathogens that address hither to unmet therapeutic needs. From this study result, it is concluded that the above Siddha medicines can be prescribed as the medicine for drug Respiratory tract infections due to pathogenic *Staphylococcus aureus, Streptococcus pneumoniae, Klebsiella pneumoniae* and *Escherichia coli.*

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