Effect of Selected Antiasthmatic Plant Constituents Against Micro Organism Causing Upper Respiratory Tract Infection

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ABSTRACT

Most exacerbations of asthma can be proven to be associated with bacterial infections and there is scientific evidence that frequent respiratory infections particularly bacterial infections provoke asthma attack. Considering these facts different plant extracts and phytoconstituents with proven anti asthmatic property had been selected for screening anti microbial activity in in-vitro models. In the present study, Coleus forskohlii Willd. extract (10% Forskolin), Piper Longum L. Extract (20% Piperine), Adathoda vasica Nees. extract (30% Vasicinone), Curcuma longa L. extract (60% Curcumin) were screened for the antibacterial activity against human pathogens causing upper respiratory infection namely Haemophilus influenzae , Streptococcus pneumoniae , Streptococcus pyrogene and Staphylococcus aureus, by taking Gentamycin , Optochin , Bacitracin and Amoxicillin as reference standards. Except for Adathoda vasica Nees. extract, all the other selected plant extracts exhibited a moderate activity antibacterial activity against selected strains .

Key words: *Curcuma longa, , Piper longum, Coleus forskohlii, Adathoda vasica.*

INTRODUCTION:

Respiratory infections with Bacteria and allied organisms, such as Mycoplasma and Chlamydia species are frequent causes of exacerbations of asthma, particularly in children, and thus they are important triggers aggravating bronchial asthma[1]. Respiratory pathogens provoke asthma attacks, although little is known about the mechanisms involved and the reason for some pathogens to be more potent inducers of wheeze than others[2]. Respiratory syncytial bacterial infection and Chlamydia infection have been proposed as possible causes of asthma. The use of macrolide antibiotics should be reserved for children in whom there is a high suspicion of atypical pneumonia, they should certainly not be used routinely to treat acute asthma [3]. Guidelines for the use of antibiotics in acute upper Respiratory tract infections clearly emphasize the minimal use of antibiotics [4].

It states that:

- Never prescribe antibiotics for simple coughs and colds.
- Never prescribe antibiotics for viral sore throat.
- Limit prescribing antibiotics over the phone to exceptional cases.
- Educate patients about the indications for antibiotic use and the risks to them of inappropriate antibiotic use.

The responsible approach is to educate patients about asthma care, to emphasize the minimum use or non-use of antibiotics and to emphasize on use of proven antiasthmatic phytoconstituents namely Curcuma longa extract (60% Curcumin) [5], Piper Longum Extract (20% Piperine)[6], Adathoda vasica extract (30% Vasicinone) [7], Coleus forskohlii extract (10% Forskolin)[8] as an alternative therapy. Hence an attempt had been made to evaluate the effect of widely used Ayurvedic antiasthmatic herbal extracts on organism causing upper respiratory tract infection.

MATERIALS AND METHOD:

Plant Extracts:

Coleus forskohlii Extract (10% Forskolin), Piper Longum Extract (20% Piperine), Adathoda vasica Extract (30% Vasicinone), Curcuma longa extract (60% Curcumin) were obtained from Sami labs Pvt.Ltd Bangalore, along with spectral data to confirm its purity. Further the extract was subjected to UV and IR spectroscopic studies to confirm the purity of the samples. The study was conducted in the Department of Biochemistry, PSG Institute of Medical Science and Research, Coimbatore.

Inoculum:

The test organisms were obtained from the patients admitted at PSG Hospital,Coimbatore.The organisms selected for the study are Haemophilus influenzae , Streptococcus pneumoniae , Streptococcus pyrogene and Staphylococcus aureus.All the isolated organisms were identified by gram staining and by performing various biochemical analysis

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Media:

1). Blood Agar Media [9]:

Blood Agar Media is used to isolate fastidious organisms like Streptococcus pneumoniae, Streptococcus pyrogene and Staphylococcus aureus.

Composition: Nutrient Substrate (20%), Sodium chloride (5%), Agar (15%),

Blood (80ml).

Preparation and Storage: Suspended 40g/litre of the media in sterile distill water,

autoclaved and cooled to 45°C.8% defibrinated blood was added and mixed well. Clear yellowish-brown medium turns to blood red color after the addition of blood.

Blood agar media plates can be stored for a maximum of 3 months in the refrigerator.

2). Chocolate Agar Media [10]:

A type of blood agar in which the blood cells have been lysed by heating the cells to 56°C.Chocolate agar is used for growing fastidious respiratory bacteria, such as Haemophilus influenzae.

Preparation of Boiled Blood Agar (Chocolate Agar Media):

To the culture media, blood is added and heated for about 10 minutes at 80°C with frequent swirling until it turns chocolate brown in color.

3).Antibacterial screening [11]:

Invitro antibacterial activities were carried out for the selected anti asthmatic plant extracts by Disc diffusion method, against human pathogens (Streptococcus pneumoniae, Streptococcus pyrogene, Staphylococcus aureus and Haemophilus influenzae) causing upper respiratory tract infection.

Streptococcus pneumoniae, Streptococcus pyrogene and Staphylococcus aureus isolated from human sputum sample were inoculated in the blood agar and human sputum isolate Haemophilus influenzae was cultured in chocolate agar medium.

The bacterial inoculum was spread by glass spreader until totally absorbed in agar layer for the development of uniform bacterial growth.

For screening, sterile 6-mm diameter discs were made from Whatman filter paper no.1 with the help of punching machine, were impregnated with the selected plant extracts (500 µg/disc), air dried and then placed in agar medium.

The antibacterial Optochin (10µg/disc), Bacitracin (10µg/disc), Amoxicillin (10 g/disc) and Gentamycin (10µg/disc) were used as reference standards. Petri plates were incubated for 18-24 hours at 30°C for development of visible bacterial growth. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the disc indicate the presence of antibacterial activity. Zone of inhibition was measured from the edge of the disc to the clear zone in millimeter. The experiments were repeated three times and the data's were expressed as the average values.

EFFECT OF SELECTED ANTIASTHMATIC PLANT CONSTITUENTS AGAINST MICRO ORGANISM CAUSING UPPER RESPIRATORY TRACT INFECTION

TABLE: 1

Sample/Standard	Conc. (µg/disc)	Zone of Inhibition of sample in mm			
		Plate A	Plate B	Plate C	Plate D
Curcuma longa extract	500	18 ± 0.2	18 ± 0.1	16 ± 0.05	14 ± 0.05
Piper Longum Extract	500	14 ± 0.05	10 ± 0.1	15 ± 0.1	12 ± 0.1
Coleus forskohlii Extract	500	10 ± 0.2	20 ± 0.1	12 ± 0.05	11 ± 0.05
Adathoda vasaka Extract	500	11 ± 0.05	N.A	10 ± 0.05	11 ± 0.05
Gentamycin	10	22 ± 0.1	-	-	-
Optochin	10	-	15 ± 0.1	-	-
Bacitracin	10	-	-	13 ± 0.05	-
Amoxicillin	10	-	-	-	26 ± 0.1

Means of 3 Values ± S.E.M.

A- *Haemophilus influenzae* on chocolate agar media.,**B-** *Streptococcus pneumoniae* on blood agar media, **C-** *Streptococcus pyrogene* on blood agar media & ***D-Staphylococcus aureus*** on blood agar media.

4). Minimal inhibitory concentration (MIC) [12]:
In experimental terms MIC is the concentration of the drug present in the last clear tube, i.e. the tube having the lowest antibiotic concentration in which growth is not observed. In the present study , series of test tubes were prepared containing the same volume of medium inoculated with the test organism (the inoculums may vary from 10^3 to 10^6 cells per milliliter).Decreasing concentration of drug were added to the tubes, Usually a stepwise dilution by a factor of 2 (two fold serial dilution.). The cultures were incubated at 30°C for 24 hours .The tubes are inspected visually to determine the growth of the organism indicated by turbidity.

RESULTS:
The four plant extracts and the four phytoconstituents namely Coleus forskohlii Extract (10% Forskolin), Piper longum Extract (20% Piperine), Adathoda vasaka Extract (30% Vasicinone), Curcuma longa extract (60% Curcumin) were screened for the antibacterial activity against human pathogens causing upper respiratory infection namely Haemophilus influenzae on chocolate agar media, Streptococcus pneumoniae, Streptococcus pyrogene and Staphylococcus aureus on blood agar media using the standard antibiotics namely Gentamycin (10 µg/disc), Optochin (10 µg/disc), Bacitracin(10 µg/disc) and Amoxicillin (10 µg/disc) respectively. It was observed that (table: 1) the extracts showed a moderate anti bacterial activity at the concentration ranging from 200 500 µg/ml against all tested organisms. It was also observed that curcuma longa extract exhibited a significant activity which was comparable with that of the standard.

CONCLUSION:
Thus the study concludes stating that the selected plant based anti asthmatic constituents namely Coleus forskohlii extract (10 % Forskolin), Piper longum extract (20 % Piperine), Curcuma longa extract (60 % Curcumin), Adathoda vasica extract (30 % Vasicinone), could be utilized in alternate anti asthmatic therapy. Since their proven anti oxidant [13] and anti microbial properties could increase the medicinal value and may help thwart symptoms of the lung diseases which is an added advantage for treating bronchial asthma. The results obtained are encouraging for further in vivo studies with an aim of obtaining clinically useful results.

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