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Research Article

SCREENING OF COMMON SIDDHA FORMULATIONS FOR ANTIMICROBIAL ACTIVITY AGAINST RESPIRATORY PATHOGENS

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ABSTRACT

Objective: The objective of this study was to screen the antimicrobial potential and minimal inhibitory concentrations (MICs) of selected commonly used Siddha formulations against respiratory pathogens.

Methods: The most frequently and clinically used Siddha formulations for respiratory infections, namely Gowri chinthamani (R1), Sivanar amirtham (R2), Poorana chandrodayam (R3), Thalaga parpam (R4), Pavala parpam (R5), and Vasantha kusumasura mathirai (R6) were screened for antimicrobial activity against microbial type culture collection strains of *Neisseria mucosa*, *Klebsiella pneumonia*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and *Aspergillus niger*, respectively, by agar well diffusion method. Tetracycline and fluconozole were used as positive control for bacterial and fungal pathogens, respectively. The zone of inhibition and the MICs were determined.

Results: Among these formulations, Sivanar amirtham (R2) and Pavala parpam (R5) showed microbial sensitivity against all the above tested respiratory pathogens. The drug Sivanar amirtham (R2) showed higher zone of inhibition when compared to the control tetracycline.

Conclusion: The study, therefore, supports the clinical claims of Siddha formulations to have potent antimicrobial activity and offer profound therapeutic benefits in respiratory infections.

Keywords: Siddha, Sivanaramirtham, Pavala parpam, Antibacterial activity, Antifungal activity, Respiratory infections.

INTRODUCTION

Respiratory infections place a huge burden in terms of disability and premature mortality, particularly in very young and elderly and those with comorbid conditions. Over 2 million fatalities per year are due to bacterial infections worldwide, and the mortality of some multidrug resistant infections has reached an alarming rate of 50-80%. Every year, India is predicted to have over 700 million episodes of acute respiratory infection and nearly 52 million episodes of pneumococcal respiratory infections [1].

Emerging antimicrobial resistance against antibiotics

Antibiotics are commonly prescribed for respiratory infections in primary care for adults and children [2]. Throughout the history, they have significantly contributed to the control of infectious diseases that were the leading causes of morbidity and mortality of human race [3]. After the decline in the discovery of new classes of antibiotics since the golden era of discovery of novel antibiotics during 1950-1970, the conventional advance for the progress of new drugs to compete the emerging and re-emerging microbial resistance to antibiotics has been the modification of existing antibiotics [4] but even new antimicrobial agents were limited by their short life expectancy [5]. A some of the credible approaches to tackle this alarming situation of antimicrobial resistance would be the exploration of ecological riches such as the marine environment, borrowing antimicrobial peptides and compounds from animals and plants mimicking the natural lipopeptides of bacteria and fungi [6].

Significance of Siddha medicine

The Siddha system of medicine owes its origin to the pre-antibiotic era whose enormous collection of classical literature, has in store a number of herbal, metallo-mineral, aquatic and animal products that are spectacular in the prevention and treatment of respiratory infections. The medicines are selected based on their ability to pacify the deranged humors vatham, pitham, and kabam pertaining to the signs and symptoms of disease, and they are anticipated to mold our immune system and provide an unsuitable environment for the growth of pathogens. Although these natural compounds cannot be claimed as antibiotics, since their antimicrobial action is not precisely validated; the presence of various bioactive compounds in plant-derived drugs are expected to have systemic action on respiratory ailments which includes anti-allergic, anti-inflammatory, expectorant and immunemodulatory properties.

The novelty of the study is to screen the antibacterial and antifungal potential of these selected Siddha classical formulations (Table 1) that are mentioned in Siddha Vaidhya Thirattu and also to provide a scientific rationale for its usage and also to support the traditional claims that are being widely used by Siddha practitioners in both government and private sectors for the treatment of commonly occurring respiratory ailments.

METHODS

The herbal and herbo-mineral Siddha formulations which are classical Siddha formulations have a wide usage in clinical practice. These preparations Gowri chinthamani (R1), Sivanar amirtham (R2), Poorana chandrodayam (R3), Thalaga parpam (R4), Pavala parpam (R5), and Vasantha kusumasura mathirai (R6) have herbo-mineral; metallic and marine compounds were market samples procured from in and around Chennai. The *in-vitro* antimicrobial study was conducted at the AUKBC Centre for Indian System of Medicine, Anna University, MIT Campus, Chennai, Tamil Nadu.

Cleaning and sterilization

The Glasswares used in the present study were cleaned with cleaning solution and sterilized in hot air oven to 180°C for 3 hrs. All nutrient media were sterilized by autoclave (121°C, 15 psi for 15-20 minutes).

Culture of pathogens

The microbial strains investigated were the *Neisseria mucosa* (MTCC 1772), *Klebsiella pneumonia* (MTCC 9751), *Streptococcus pneumonia* (MTCC 655), *Staphylococcus aureus* (MTCC 96), *Pseudomonas aeruginosa* (MTCC 10462), and *Aspergillus niger* (MTCC 281) were

Table 1: Detailed information on selected Siddha formulations

S.No	Study drugs	Chief ingredients	Indications
R1	Gowri chinthamani	Mercury, sulfur, Borax	Kasaswasam (bronchial asthma) Irumal (cough), Kshayam (tuberculosis), Kandamalai (lymphadenitis), Suram (fever)
R2	Sivanar amirtham	Rhizome of fern, mercury, sulfur, red orpiment, borax, *Aconitum ferox, Piper nigrum, Piper longum* and *Zingiber officinale*	20 types of Kapham and 5 types of swasam (all kinds of respiratory ailments)
R3	Poorana chandrodayam	Gold, mercury, sulfur, red cotton flower	Kshayam (tuberculosis), ilai (cough), soolai (pains), suram (fever)
R4	Thalaga parpam	Thalagam, latex of calotropis	Ilai (cough), suram (fever), manthara kasam (bronchialasthma)
R5	Pavala parpam	Pavalam, lemon juice, sugar candy	Ilai (cough), mikka kabam (aggravation of phlegm), suram (fever), suvasam (difficulty in breathing)
R6	Vasanthakusumasura mathirai	Cinnabar, borax, *Syzygium aromaticum, Piper longum, Costus speciosus, Anacyclus pyrethrum, Glycerhizza glabra*	Suram (fever), kasam (cough), thummal (allergic rhinitis)

S. aromaticum: Syzygium aromaticum

purchased from MTCC, Chandigarh, India, and they were sub cultured based on their standard protocol according to the National Committee for Clinical Laboratory standard guideline. The bacterial pathogens were cultured in nutrient agar (Himedia, Mumbai) and the fungal pathogen was cultured in Sabourd's dextrose broth (Himedia, Mumbai). The pathogenic bacterial culture was inoculated into the sterile nutrient broth and incubated at of 25°C for 48-72 hrs until the culture attained a turbidity of 0.5 McFarland units.

Agar well diffusion assay

The extracts were tested for antibacterial activity using agar well diffusion on solid media [7]. The agar was poured into the assay plate of 9 cm in diameter. 100 µl of 500 mg tetracycline was pre-inoculated onto the plates to prevent the growth of bacterial contamination and allowed to cool down on a leveled surface. Once the medium was solidified, four wells, each 6 mm in diameter were cut out of the agar, and 50 µl of the compounds were filled into each well at different concentrations. Tetracycline and fluconozole were used as positive control. The zone of growth inhibition for each extract was measured from agar well to the end of the zone in millimeter. The minimal inhibitory concentrations (MIC) of the compounds were also determined. Triplicates were maintained.

RESULTS

The zone of inhibition and MIC was determined as shown in Fig. 1. Among the selected Siddha formulations, the sample Gowri chinthamani (R1) showed antimicrobial activity against Gram-negative organisms (*N. mucosa*, *K. pneumonia*, *P. aeruginosa*). The formulations Sivanar amirtham (R2) and Pavala parpam (R5) showed broad spectral antibiotic activity against all the tested Gram-positive and Gram-negative organisms of which the drug Sivanar amirtham showed higher zone of inhibition than the positive control against the pathogens *N. mucosa*, *K. pneumonia*, *S. aureus*, and *P. aeruginosa*. The sample Vasantha kusumasura mathirai (R6) showed specific antibacterial activity against *S. aureus* and *P. aeruginosa*. The samples Poornachandrodayam (R3) and Thalaga parpam (R4) did not show antimicrobial effects against any of the tested pathogens. Except the samples Poornachandrodayam (R3) and Thalaga parpam (R4), all other samples showed the anti-fungal effect, but the zone of inhibition was comparatively lesser than that of the control (fluconazole).

Determination of MIC

For MIC, two-fold serial dilutions of the extracts were performed. Each inoculum was prepared in its respective medium and density was adjusted to 0.5 McFarland standard and diluted to 1:100 for the broth microdilution procedure. Micro-titer plates were incubated at 370 and MIC was recorded after 24 hrs. The MIC is the lowest concentration of the compound at which the microorganism tested does not demonstrate visible growth. The lowest MIC values were obtained from the drug Sivanar amirtham (R2) (Table 2).

Table 2: MIC of Siddha formulations

Microbial strains	R1	R2	R5	R6
MIC (µg/ml)				
N. mucosa (MTCC 1772)	100	50	200	-
K. pneumonia (MTCC9751)	150	60	200	-
S. pneumonia (MTCC65)	-	75	150	200
S. aureus (MTCC 96)	-	100	175	-
P. aeruginosa (MTCC10462)	150	75	200	175

MIC: Minimal inhibitory concentration, *N. mucosa: Neisseria mucosa, K. pneumonia: Klebsiella pneumonia, S. pneumonia: Streptococcus pneumonia, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa*

DISCUSSION

The organisms that were included in the study consisted of both Gram-positive (*S. pneumonia*, *S. aureus*) and Gram-negative (*N. mucosa*, *K. pneumonia*, *P. aeruginosa*) and a fungal pathogen *A. niger*. *S. pneumonia* is the most common bacterial cause of upper and lower respiratory tract infections, particularly pneumonia [8]. The clinical symptoms of pneumonia include a cough, breathlessness, inability to feed, increased respiratory rate, in the drawing of intercostal space, fever and tachycardia [9]. Pneumonia also exhibits non-respiratory symptoms such as confusion, headache, myalgia, nausea and abdominal pain. *S. aureus* and *P. aeruginosa* are particularly prominent causes of infections in cystic fibrosis and destroys the lung parenchyma leading to respiratory failure [10]. *Streptococcus aureus* sets the stage for lower respiratory tract infections [11] which include bronchitis, bronchiolitis, pneumonia with symptoms of cough, fever, chest pain and wheezing [12]. *N. mucosa* is a part of normal human naso pharyngeal flora and infrequently causes infections [13]. *K. pneumonia* is the third most common bacterial cause of hospital acquired pneumonia in immunecompromised patients [14]. *P. aeruginosa* most commonly affects the lower respiratory tract in humans and can range in severity from colonization to severe bronchopneumonia [15].

The drug Sivanar amirtham is a popular herbo-mineral Siddha classical formulation and has been claimed in the Siddha literature to treat 20 types of kapham and 5 types of swasam diseases which includes all kinds of respiratory ailments of both allergic and infectious origin. The ingredients mercury and sulfur are purified as per Siddha classical literature, and they are transformed into stable and non-toxic compounds which are therapeutically effective. Pavala parpam is specifically meant in Siddha literature for the management of respiratory symptoms such as cough, copious phlegm, fever, and breathing difficulty. It is derived from marine sources of coral reefs and calcinated (Parpam) using herbal decoctions and incineration (Pudam).

The titration of metals with herbal juices, grinding for longer duration and heat treatment diminishes the toxicity and causes the reduction

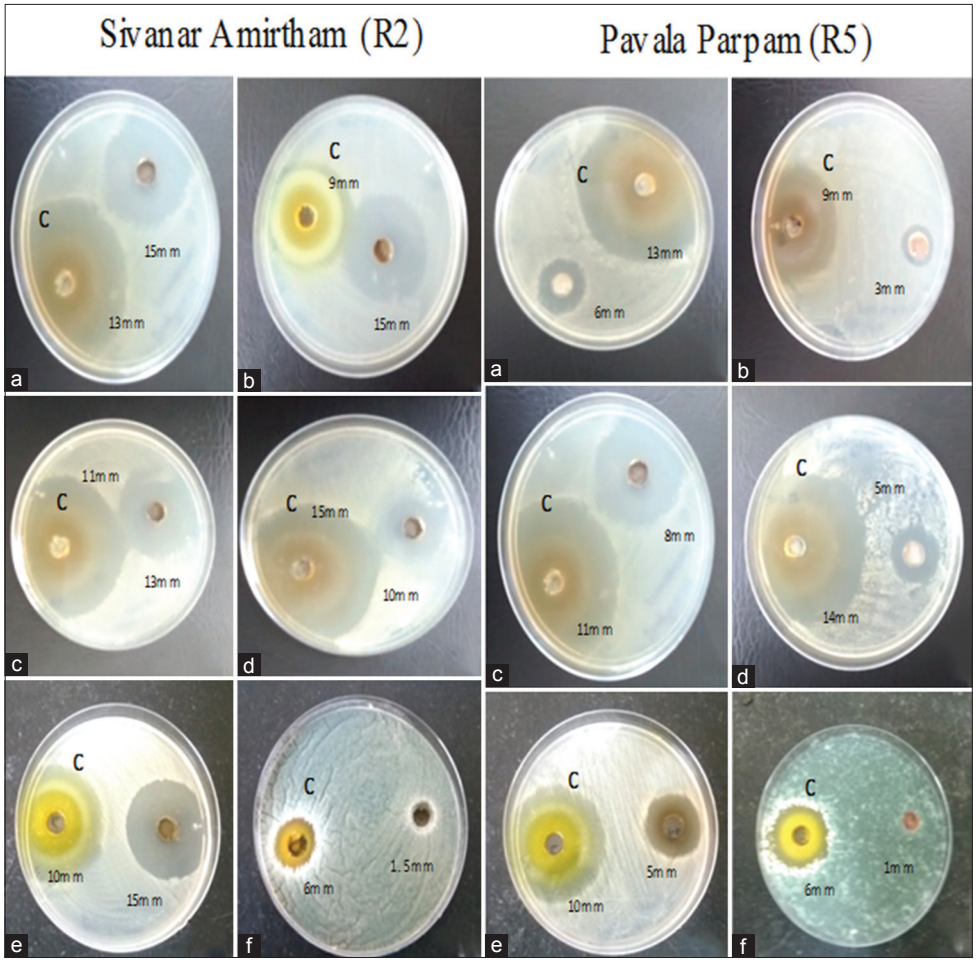

Fig. 1: Antimicrobial activity of Siddha formulations against respiratory pathogens. (a) *Neisseria mucosa*, (b) *Klebsiella pneumonia*, (c) *Streptococcus pneumonia*, (d) *Staphylococcus aureus*, (e) *Pseudomonas aeruginosa*, (f) *Aspergillus niger*

in particle size, and they are transformed to nanoparticles due to the change in their chemical structure [16]. Most of the Siddha drugs with mercury and sulfur combination forms mercuric sulfide (HgS) which is the least toxic and less absorbable form than any forms of mercury [17]. HgS has been used in traditional medicine for thousands of years. The use of mercury in therapeutics and its combination with any drug is supposed to act as a bio-enhancer by reducing its dose and to increase the efficacy manifolds. It also increases the bioavailability of the drug, thereby reducing the time required for the onset of action of the drug [18]. Further, the presence of organic matter acts as a coating material on the surface of the metallic compound and the metal compound act as a carrier of organic matter similar to the recent concepts of drug delivery system [19]. Marine microorganisms are found in symbiosis with marine sponges, corals and other species. Hence, they are a natural marine enzymes and various bioactive peptides which are antimicrobial compound and has served as a natural source of drug discovery since ancient times [20]. A study also revealed that coralline hydroxyapatite particles loaded with medically active substances improved drug stabilization, higher drug encapsulation efficiency of the carrier and showed significant ability to control the growth of bacteria [21].

Usually, both these Siddha formulations Sivanar amirtham and pavala parpam are used for respiratory ailments in very low dosages (100-200 mg) in combination with an appropriate herbal powder (Chooranam) and a suitable adjuvant. Pharmacological studies on Sivanar amirtham have reported it to have anti-inflammatory and analgesic properties [22]. Moreover, according to Siddha literature (gunapadam, thathu), the unique methodology of prolonged grinding and incineration provide a very long shelf life to these medicines when compared to the current antibiotics. As many of the popular antibiotics are nearing their expiry because of resistant microbial strains and the introduction of new compounds may cost a billion of dollars for which the lifetime is only 8-10 years in the market [23], an alternate bioavailable source to alleviate this stressful situation is being searched continuously. As India has tremendous inexhaustible natural resources, the above studies make us to understand that both these Siddha formulations can be synergistically used in clinical practice for respiratory infections as they have potent antimicrobial action, anti-inflammatory and analgesic properties besides being bio-enhancers with a longer shelf life.

CONCLUSION

Our findings suggest that Pavala parpam (R5) and Sivanar amirtham (R2) shows broad spectral antibacterial activity among the tested samples of Siddha formulations of which Sivanar amirtham showed a significant zone of inhibition. Hence, it can be acknowledged as a potent drug with antimicrobial sensitivity against these respiratory pathogens. More literature formulations have to be identified and researches may be warranted at the molecular level and also the pharmacodynamic targets of these age old Siddha medicines should be achieved to optimize clinical success in the management and prevention of respiratory infections.

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