Ancient Science of Life, Vol. 28, No. 2(2008) Pages 19-25

Comparative study on efficacy of Antimicrobial activity of three Ayurvedic drugs Balaguduchi, Dhanadanayanadi and Dasamoolabala on isolated Nosocomial Pathogens

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Abstract: *The antibacterial activity of various solvent fractions of Balaguduchi, Dhanadanayanadi and Dasamoolabala was studied against the isolated nosocomial pathogens by in vitro method. The methanol, acetone, choloroform, acetone fractions of test drugs and decoctions exhibited different levels of antibacterial activity from low to very high level against the gram positive and gram –negative bacteria. The zone of inhibition of each fraction of drugs was compared with the standard antibiotics Penicillin and Streptomycin. The present study proves and explains the ability and potency of the extracts of Balaguduchi, Dhanadanayanadi and Dasamoolabala as a preventive measure of secondary infections by bacteria in the hospitalixe patients. Even though, these medicines are prescribed in ayurveda for the different clinical indications, the possible secondary activity is also proved through this study.*

Key words: *Nosocomial pathogens, antibacterial activity, Balaguduchi, Dhanadanayanadi, Dasamoolabala.*

INTRODUCTION

The search for drugs and methods of identifying remedies for a number of aliments is as old as humanity. The various measures and devices, Practices and methods varied with the passage of time. Ayurveda, the most ancient of the sciences is not just a compendium of diseases but a system of medicine that has enunciated principles relating to promotion and maintenance of health.

Nosocomial infections are defined as infections acquired during or as a result of hospitalization. Generally, a patient who has been in the hospital for less than 48 h and develops an infection is considered to have been incubating the infection prior to hospital admission. Most infections manifested after 48h are considered to be nosocomial[1]. Patients may still develop a nosocomial infection after hospital discharge

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if the organism presumably was acquired in the hospital. Based on the sites of nosocomial infections, it can be generally classified as, Urinary tact infections, surgical sit infections, Respiratory tract inflections, Blood stream infections, Skin infections, gastrointestinal tract infections, Central nervous system infections etc[2]. Surgical wound infection developing in the weeks after hospital discharge is an example of nosocomial infection2. Nosocomial infections contribute significantly to morbidity and even mortality, as well as to excess costs, for hospitalized patients[3]. It is estimated that 3 to 5 percent of patients admitted to an acute care hospital in the United states acquire a new infection, which accounts for about 2 million nosocomial infections per year and an annual cost in excess of 2 billion dollars[4]. Some estimate that the cases of death double in patients who develop a nosocomial infection, although clearly such factors as underlying disease and severity of illness also play an important role[5]. About 30 % of patient admitted to hospitals and nursing home in the country acquire nosocomial infections as against an impressive 5% in the west, according to Members of Hospital Infection Society (HIS), India. This alarming situation is attributed to hospitals reluctance to invest in infection control, lack of awareness and improper waste management. In a few instances, nosocomial infections lead to septicemia having a mortality rate of 80%[6]. Worldwide, around 14 lakh people suffer from hospital-acquired infections[7].

Resistance to antimicrobial agents such as antibiotics is emerging in a wide variety of organisms and multiple drug resistant organisms pose serious threat to the treatment of infectious diseases 8,9,10. Plants are known to possess antibacterial activity; several of them have been used in traditional medicine to treat wound infections.

The Present study aims to evaluate the ability and efficacy of antimicrobial activity of various solvent fractions of three basic Ayurvedic drugs/formulations, Balagulichi, Dhanadanayanadhi and Dasamoolabala on the isolated nosocomial pathogens.

MATERIALS AND METHODS

MATERIALS REQUIRED:

EQUIPMENTS: Soxhlet Extraction Unit, Laminar Flow, Autoclave, Incubator, Zone Reader

MEDIA : Nutrient broth, Nutrient Agar, Blood Agar, Mueller Hinton Agar were purchased from Hi-Media, India.

SOLVENTS & CHEMICALS: Methanol, Acetone, Petroleum ether, Chloroform were purchased from Merck, India. All are AR grade. Dimethyl formamide-AR grade-Merck, Whatmann No.1. Filter Paper Discs. Paper Discs. Penicillin and Ciprofloxacin from local market.

DRUGS: Balagulichi, Dhanadanayanadhi, and Dasamoolabala were received from the Pharmacy Division of Central Research Institute (Ayurveda), Cheruthuruthy Kerala these drugs were prepared as per the Ayurvedic formulary of India 11,12.

METHODOLOGY

SOLVENT EXTRACTION:

The 25 gm of dry material of drugs Balagulichi, Dhanadanayanadhi, and Dasamoolabala was weighted and packed individually in the cellulose thimble for solvent extraction in the Soxhlet unit. The four different solvents of various polarity like methanol, Petroleum ether, Chloroform and Acetone were used for the extraction. Each solvent extraction was carried out individually using freshly medicine each time. This extraction procedure was carried out for 12 hours continuously. At the end of the extraction procedure, the solvent was removed by distillation and the Solvent-free dried extract was dissolved in Dimethyl formamide and it used for the present study at the concentration of 10 mg/ml. the water extract decoction was prepared as per Ayurvedic formulary of India. The filtered portion of decoction was used for the present study.

PREPARATION OF MEDIA:

The media plates were prepared ascetically as per the instruction given by the suppliers (Hi-Media). The solidified plated were ready for inoculation and excess plates were stored in 40C for further use.

ISOLATION OF BACTERIA:

The pathogenic bacterial strains of E.Coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Staphylococcus aureus, were isolated from the biological samples (urine, throat swab, nasal discharges, sputum) of IP patients, bystanders, coworkers of CRIA, Cheruthuruthy. The selective mediums were used for specific culturing of E.coli, Pseudomonas aeruginosa, Klebsiella pneumonia, and Staphylococcus aureus. The isolates were confirmed by specific biochemical tests. The stock cultures were prepared and stored in Nutrient-Agar medium at 40C.

INOCULATION OF TEST PLATES:

Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension to contain approximately $1\text{-}2x10^8$ CFU/ml, a sterile cotton swab was dipped into the adjusted suspension and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab.

The dried surface of Mueller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. The procedure was repeated by streaking two more times, rotating the plate approximately 60^o each time to ensure an even distribution of inoculum and as a final step, the rim of the agar was swabbed.

PREPARATION OF DISCS:

The circular discs of 6mm diameter were prepared from whatman No.1. filter paper, sterilized and used. The discs were found to have 251 holding capacity.

DISC DIFFUSION METHOD:

The Kirby-Bauer's Disc Diffusion method was used in this present study to determine the antimicrobial susceptibility of test samples. The national Committee for Clinical Laboratory standards (NCCLS) is recommending the Kirby-Bauer's disc diffusion method for performing antimicrobial susceptibility testing 13,14. The clear labeling of samples were marked on the plate. The plates were then inverted and incubated at 370C for 24 hours.

ZONE OF INHIBITION:

The zone of inhibition was obtained by measuring the clear zone around each disc by Zone Reader. The value were noted in millimeter. The statistical analysis was carried out.

RESULTS AND DISCUSSION

The overall study shows that various solvent fractions of the Ayurvedic formulations of Balaguduchi, Dhanadanayanadi and Dasamoolabala do have the potential antimicrobial activity against the nosocomial pathogens. Some of the solvent fractions do not have activity against Gram-positive but on Gramnegative and vice-versa.

The present study reveals that the Ayurvedic formulation have the specificity towards types and species of organisms and the efficacy was comparable one with standard antibiotics.

EXTRACTION OF DRUGS:

The extract efficiency of polar and non-polar solvents on each drug was determined and unextractable portion was calculated. The details have been mentioned in table1.

BALAGULICHI:

The methanol fraction of Balaguduchi was high in quantity (10.3%) among the other solvent fractions. The polar solvents brought the highest quantity of extraction (Table 1).

The antibacterial activity of each fraction of Balaguduchi has been presented in Table 2. Methanolic fraction of drug showed highest activity on Gram-positive bacteria Staphylococcus (14.1±0.76) and the lowest activity was on Klebsiella. The acetone fraction has nil activity on Klebsiella but on other microorganisms. The chloroform extract showed zone of inhibition 12.0±1.15 for E.coli followed by Pseudomonas (11.0±0.57) and Staphylococcus aureus (10.0 ±1.0). Petroleum ether fraction of drug does not have activity on Klebsiella but highest zone of inhibition on Staphylococcus (Table 2). The decoction of the drug was observed to be effective in E.Coli and Staphylococcus but ineffective in inhibiting the growth of Klebsiella and Pseudomonas. The antimicrobial potency of the various fractions of the drug was compared with other experimental study dugs Dhanadanayanadi and Dasamoolabala.

DASAMOOLABALA:

The overall extractable portion was very low in the drug Dasamoolabala. The methanol and acetone fraction found to be 4.31% and 1.21% respectively. The chloroform fraction was 0.78% and 0.62% for Petroleum ether.

Extraction percentage of Balaguduchi, Dhanadanayanadi and Dasamoolabala

S.No.	Name of Solvent	Extract obtained (in Percentage)		
		BALAGUDUCHI	**DHANADHANAYANADI**	**DASAMOOLABALA**
1.	Methanol	10.3	15.84	4.31
2.	Acetone	7.42	11.2	1.21
3.	Chloroform	7.0	4.48	0.78
4.	Petroleum Ether	4.24	3.25	0.62
5.	Unextractable Portion	71.04	65.23	93.06

Table 1. The details of extraction efficiency (in percentage)
of Balaguduchi, Dhanadhanayanadi and Dasamoolabala using Solvent Extraction Method.

Antimicrobial activity of Balaguduchi

S.No.	Name of Sample	Zone of Inhibition (in mm) on microbes isolated from Biological Samples			
		E.coli	**Klebsiella**	**Pseudomonas**	**Staphylococcus aureus**
1.	Methanol Extract	9.8 ± 1.04	9.1±1.25	10.1±1.04	14.4±0.76
2.	Acetone Extract	10.8±1.6	NIL	8.8±0.76	11.1±1.0
3.	Chloroform Extract	12.0±1.15	NIL	11.6±0.57	10.0±1.0
4.	Petroleum Ether Extract	11.8±1.04	NIL	10.1±1.04	16.3±1.52
5.	Decoction	10.1±1.08	NIL	NIL	8.3±0.57
6.	Penicillin	Not Used	Not Used	Not Used	20.0±0.35
7.	Ciprofloxacin	33.0±1.06	35.0±0.75	41.0±0.70	Not Used

Table.2 Antimicrobial activity of different solvent extractions of BALAGUDUCHI
at 250 µg/disc concentration and comparison with standard antibiotics
at 25 µg/disc concentration level. (Values are expressed as MEAN±SD)

Antimicrobial activity of Dhanadanayanadi

S.No.	Name of Sample	Zone of Inhibition (in mm) on microbes isolated from Biological Samples			
		E.coli	**Klebsiella**	**Pseudomonas**	**Staphylococcus aureus**
1.	Methanol Extract	NIL	NIL	11.1±0.76	11.3±0.75
2.	Acetone Extract	NIL	NIL	9.1±1.25	14.5±0.5
3.	Chloroform Extract	11.5±0.50	NIL	10.7±1.04	11.5±0.76
4.	Petroleum Ether Extract	NIL	NIL	12.1±1.04	9.5±0.86
5.	Decoction	12.1±1.05	13.8±1.04	8.8±0.76	10.5±0.5
6.	Penicillin	Not Used	Not Used	Not Used	20.0±0.35
7.	Ciprofloxacin	33.0±1.06	35.0±0.75	41.0±0.70	Not Used

Table.3.Antimicrobial activity of different solvent extractions of DHANADANAYANADI at 250 µg/disc concentration and comparison with standard antibiotics at 25 µg/disc concentration level. (Values are expressed as MEAN±SD)

Antimicrobial activity of Dasamoolabala

S.No.	Name of Sample	Zone of Inhibition (in mm) on microbes isolated from Biological Samples			
		E.coli	**Klebsiella Pneumoniae**	**Pseudomonas aeruginosa**	**Staphylococcus aureus**
1.	Methanol Extract	11.1±1.04	9.8±0.76	11.8±0.76	10.0±1.0
2.	Acetone Extract	11.3±0.57	11.5±0.5	8.6±0.57	11.0±1.20
3.	Chloroform Extract	9.8±0.76	13.8±0.76	9.1±1.25	10.3±1.04
4.	Petroleum Ether Extract	14.0±1.0	8.8±0.76	10.8±0.76	NIL
5.	Decoction	9.8±0.76	9.1±0.06	NIL	NIL
6.	Penicillin	Not Used	Not Used	Not Used	20.0±0.35
7.	Ciprofloxacin	33.0±1.06	35.0±0.75	41.0±0.70	Not Used

Table.3.Antimicrobial activity of different solvent extractions of DASAMOOLABALA at 250 µg/disc concentration and comparison with standard antibiotics at 25 µg/disc concentration level. (Values are expressed as MEAN±SD)

The methanolic fraction of Dasamoolabala showed good antibacterial activity against E.coli and Pseudomonas and minimal activity on Klebsiella and Staphylococcus. Both fractions of acetone and chloroform exhibited antibacterial activity on all the Gram positive and Gram negative study microorganisms. The zone of inhibition was higher in Klebsiella against acetone and chloroform. Decoction of drug does no inhibit the growth of Pseudomonas and Staphylococcus but on E.coli (9.8±0.76) and Klebsiella (9.1±1.06). the standard antibiotics Penicillin and Ciprofloxacin were used in the study as a control at the concentration of 25µg/disc. Penicillin was observed to have zone of inhibition 20.0 ± 0.55 on Staphylococcus and Ciprofloxacin exhibited 33.0 ± 1.06, 35.0±0.75 and 41.0±0.70 on E.Coli, Klebsiella and Pseudomonas respectively.

The experiment exhibited the antibacterial property of the ayurvedic drugs/formulations Balaguduchi, Dhanadanayanadi were having nearly 30 to 40% of extractable portion and in this 60 to 75% were by polar solvents. The carbohydrates, proteins, pectins, cell wall molecules, lignans might have come with the polar solvents like methanol and acetone. The fatty acids and lipids might have come with the non-polar solvents and so the quantity was also very low comparing with earlier. But both of the extracts were found to have significant antibacterial activity. The novelty of the study is that the experiment proves and explains the ability and potency of the extracts of Balaguduchi, Dhanadanayanadi and Dasamoolabala as a preventive measure of the seconday infections by the bacteria in the hospitalized patients for the treatment of various chronic diseases (primary disease) such as paralysis, rheumatoid arthritis etc. Since the secondary infections are mainly associated with the environment, food habits and mostly air-borne diseases, these extracts can be used as a preventive medicine, such as vaccines in modern medicine, especially to the patients, bystanders, other staffs those who are associated with the hospital work .

The study made to understand that even though the decoction of some drugs does not have the antibacterial property against some pathogens, but fractionation of the same drug by suitable solvent may have the property. Further purification of these extracts through the various advanced scientific methodologies, may bring out variety of novel molecules that are present in the valuable Ayurvedic drugs, and identification of these molecules will be helpful to use as biomarkers of these drugs. Since these cruds extract itself, has the comparable antibacterial activity with the Penicillin and Ciprofloxacin, the further purification and fractionation of these crude extracts, certainly will bring the multifold functional ability – molecules that will have greater biopotency than the drugs presently existing in the modern pharmacopoeia. So the present study revealed the new functional properties of the Balaguduchi, Dhanadanayanadi and Dasamoolabala drugs with reference to preventive measure of nosocomial infections. The further research is highly essential to identify and characterize the various functional molecules of these drugs and their potency also, for the welfare of human beings.

ACKNOWLEDGMENT

The authors are graceful to the Director, CCRAS, New Delhi for the constant support and direction. Thanks are also due to all other staff members of CRIA, Cheruthuruthy, those who helped in the accomplishment of the study.

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