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RESEARCH ARTICLE

ANTIBACTERIAL EFFECT OF TRIPHALA CHURNA AGAINST COMMON WOUND PATHOGENS

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ABSTRACT

Background: The increasing overuse and sometimes, failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic infectious microbes, have led to the screening of several medicinal plants for their potential antimicrobial activity. The antibacterial effect of cold and hot extract of *Triphalachurna* is one such medicinal extract.

Materials and Methods: The clinical samples received at the Department of Microbiology, Kasturba Hospital, Manipal were tested using the extract by agar dilution method against common wound pathogens such as MSSA, MRSA, *E. coli* (probable ESBL producer), *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter.*

Results: *Triphalachurna* was found most potent against all the organisms. The study showed susceptibility of wound pathogens to the aqueous extract of *Triphalachurna*. The cold extraction was proportionally effective against each Gram negative organism in comparison to hot extraction. *Acinetobacter* species were the most susceptible to the antibacterial effect, followed by *P. aeruginosa* which showed intermediate susceptibility. *E. coli* and *K. pneumoniae* showed least susceptibility. MRSA was more susceptible than MSSA against the cold extraction; hot extraction was equally effective against both MRSA and MSSA.

Conclusion: The herbal preparation helps in inhibiting the growth of pathogenic bacteria and hence preventing wound infection.

INTRODUCTION

India has an ancient heritage of traditional herbal medicine. *Triphala* is one of the most popular and useful medications, available in Indian medical pharmacopeia as a "churna", a finely sieved powder used as a source of medicinal agent to cure many infections. As the term suggests, it is a combination of three potent herbal fruits- *Terminaliachebula*, *Terminaliabellerica and Teminaliemblica* (Yogesh *et al.,* 2010). Researchers have found that this medicinal combination is a potent antioxidant along with additional antimicrobial and anti-cancerous properties (Jagetia *et al.,* 2002; Rege *et al.,* 1999). Both aqueous and alcoholic extractions of *Triphala* exhibit a broad spectrum antimicrobial activity against the microorganisms from human secretions. Aqueous extracts shows better activity than ethanolic extract for all strains (Mehta *et al.,* 1993). Infection is the major problem in the management of wounds where alcoholic extract of *Triphala* is known to produce both antimicrobial and wound healing activity (Jagetia *et al.,* 2002).

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One of the studies shows the eradicating potential of *Triphala* against *E. faecalis* biofilm formed on tooth substrate (Kaur *et al.,* 2002). It is also tested for its total antioxidant activity and antibiotic assay against *Streptococcus mutans*. The ethanolic herbal extract effectively inhibits the biofilm formation and better antioxidant activity is exhibited (Kumar *et al.,* 2008). Nearly 60-70% of the children in in Indian child populations suffer from dental caries. Mouth rinsing is the most cost effective method of preventing dental caries. Studies also have determined the equivocal effect of *Triphala* mouth-wash in prevention of dental caries in comparison to chlorhexidine (Premila, 2006).

Conventional drugs usually provide effective antibiotic therapy for bacterial infections, but due to an increasing problem of antibiotic resistance; there is a need for new solutions. Herbal preparations are preferred to synthetic antibiotics in reducing development of resistance (Deivy Clementino de Lima *et al.,* 2005). Herbal medicines are being used increasingly as dietary supplements to prevent most of the health-related complications, including infections (Yogesh S Biradar *et al.,* 2008). Many herbal products claim to have antimicrobial

properties but only few studies have been tried to prove these. Antibacterial activity of *Triphala* has been studied both *in-vivo* and *in-vitro* experiments. *Triphala* is used commercially as an ointment, mouth-wash, toothpaste, etc.

In-vitro studies to demonstrate antibacterial activity of the *Triphala* aqueous extract against wound pathogens have not been reported in literature. So, the present study was planned to test the antimicrobial property of *Triphala* aqueous extract against common wound pathogens.

MATERIAL AND METHODS

Bacterial wound pathogens: Isolated wound pathogens were collected from clinical samples obtained at the Department of Microbiology, Kasturba Hospital, Manipal. They were characterized through microscopic examination, Gram staining, biochemical test profile and analyzed for drug resistance using standard antibiotics. Standard control strains were also used. Methicillin-resistant *Staphylococcus aureus* (MRSA), Methicillin- sensitive *Staphylococcus aureus* (MSSA) D-positive, *Pseudomonas aeruginosa*, *Klebsiellapneumonia, Escherichia coli* (ESBL producer) and Acinetobacter *spp* were included in the study.

Inclusion criteria: Only the above mentioned bacterial wound pathogens isolated from clinical samples were included in the study

METHODOLOGY

Preparation of aqueous extract of Triphala

Antibacterial agent

Triphalachurna (crude powder) obtained from *Zandu*, India, at net weight of 60gm per bottle with 333.3mg per fruit of *Terminaliachebula, Terminaliabellerica, Phyllanthusemblica* was used as raw source. Cold extraction: 30gms of *Triphala* powder was taken in a cloth bag and immersed in 50ml of distilled water. One drop of chloroform was added to prevent fungal growth. Preparation was kept for 24 hours with occasional stirring. At the end of 24 hours, the cloth bag was removed and pressed so as to collect as much of extract as possible. The water extract was evaporated over a water bath until a semisolid or powdery mass was obtained. The concentrated extract obtained was lyophilized and used for the study.

Hot extraction method: 30 gm of crude powder was boiled intermittently in round bottom flask with 50ml of water for 10 minutes. Water extract was evaporated over a water bath in a china dish. The semisolid powdery mass obtained was dried over a water bath and obtained hard mass of extract was powdered using a mortar and used for the study.

Preparation of Bacterial suspension

Isolated bacterial pathogens and control strains culture were grown overnight in sterile peptone water and their optical density was adjusted to 0.5 McFarland turbidity standard.

Antimicrobial testing by agar dilution method using Triphala extract

Test plates were prepared using Muller Hinton agar with the concentration from 50µg till 3000 µg concentration (about 30 different concentrations) of Triphala cold and hot extract respectively. A standardized suspension of test bacteria and controls were inoculated by spot inoculation method onto a series of these agar plates and incubated at 37^{o}C for 24 hours and results were recorded.

Kirby- Bauer disk diffusion method is used to study the susceptibility of organisms to various drugs and zone of inhibition was observed and recorded.

Statistics

Pattern of inhibition at different concentration of *Triphala* cold extract and hot extract by the six common bacteria was compared.

RESULTS

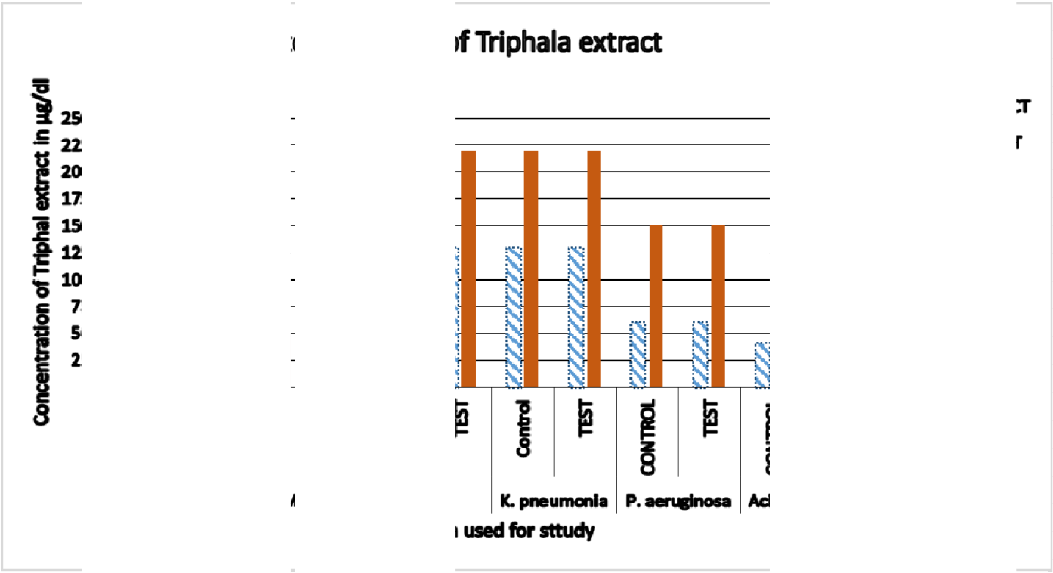
The cold extraction was proportionally effective against each Gram negative organism in comparison to hot extraction. *Acinetobacter* spp. were more susceptible to the antibacterial effect of *Triphala. E. coli* and *K. pneumonia* showed less susceptibility compared to *P. aeruginosa* which showed intermediate susceptibility in comparison to *Acinetobacter* spp. and Lactose fermenting bacilli (*E. coli* and *K. pneumonia*). Among Gram positive organism MRSA is more susceptible than MSSA against the cold extraction; hot extraction is equally effective against both MSSA and MRSA (Table 1 & 2).

Antibiotic sensitivity test done using chemotherapeutic drugs by disk diffusion method showed the resistance pattern of these organisms against the drugs used. E.g. MRSA, MSSA resistant to cefoxitin, probable ESBL producing E coli, *K. pneumonia* and *Acinetobacter* species were showing resistance to almost all antibiotics used. Only *P. aeruginosa* was sensitive to all the drugs used.

Antibiotic susceptibility against following chemotherapeutic drugs:

Table1. MSSA and MRSA antibiotic susceptibility test (AST) pattern to the drugs used

Antibiotics	MSSA	MRSA
Amox-clav	Sensitive	Resistant
Amp/Amox	-	Resistant
Cefazoli	Sensitive	-
Cefuroxime	Sensitive	-
Ciprofloxacin	-	Resistant
Cefoxitin	Sensitive	Resistant
Cotrimoxazole	Sensitive	Resistant
Gentamicin	Sensitive	Resistant
Erythromycin	-	Resistant
Tetracycline	-	Resistant
Clindamycin	Sensitive	Resistant



Graphical representation:Concentrations of Triphala extract at which growth of organisms were inhibited (Result of Hot and Cold extraction for control and Test organisms)

Table 3. ***E.coli, Klebsiella, Acinetobacter and P. aeruginosa showing*** **AST pattern**

Antibiotics	E.coli-	K.pneumoniae	Acinetobacter	P.aeruginosa
Amikacin	-	Sensitive	Sensitive	Sensitive
Amp/Amox	-	Resistant	-	-
Cefuroxime	-	Resistant	-	-
Amox-clav	Resistant	Resistant	-	-
Cefotaxime	Resistant	Resistant	-	-
Ceftazidime	-	-	Sensitive	Sensitive
Ciprofloxacin	-	Resistant	Sensitive	Sensitive
Cotrimoxazole	-	Resistant	-	-
Gentamicin	-	Resistant	Resistant	Sensitive
Netilmicin	-	Sensitive	Resistant	Sensitive
Piperacillin	-	-	Resistant	Sensitive
Tobramycin	-	-	Resistant	Sensitive
Cefoperazone-Sulbactam	Sensitive	Sensitive	Sensitive	-
Imipenem/meropenem	Sensitive	Sensitive	Resistant	-
Piperacillin-Tazobactam	-	Sensitive	Sensitive	-

DISCUSSION

Antibacterial effect of both cold and hot extract of *Triphalachurna* against 6 common organisms showed sensitivity and resistant patterns to the antibiotic drugs. Cold extract of Triphala was found to be more effective than the hot extract. The probable reason could be that the heat generated during hot extraction destroyed the elements in the extract which could act as an antibacterial agent. Gram positive organisms were more susceptible than gram negative organisms. Difference in the cell wall composition of these organisms could have affected these results. On comparing our results with other chemotherapeutic drugs, the organisms which were seemingly resistant to other drugs were found to be susceptible to the *Triphala* extract. In this study, we used aqueous extract of *Triphala*, study could be modified further using alcoholic extract which could provide better results and can be compared with aqueous extracts. The test can be done against clinical specimens of all type of bacterial infections such as respiratory infections, urinary infections, GIT infections etc. or the organisms which are highly resistant to chemotherapeutic drugs and also against fungal agents. Agar dilution method was used to test the antimicrobial effect. The test can be done using disc diffusion method by impregnating the filter paper disc into the extract and standardizing the quantity of extract and the test inoculum. In vivo study can be done to test the antimicrobial effect of Triphala by washing the wound with extract or applying the extract on affected area and recording the results or using animal study. Using many medicinal extract one can effectively treat bacterial infections and can avoid the major problem of antibiotic resistance exhibited by bacteria against chemotherapeutic agents.

Conclusion

The increasing failure of chemotherapeutics and extremes of an antibiotic resistance exhibited by pathogenic microbial infectious agents, have led to the screening of several medicinal plants for their potential antimicrobial activity. The study shows the susceptibility of wound pathogens to the aqueous extract of *Triphalachurna* which combines the antimicrobial effect of all three fruits, P. emblica, T. chebula

and T. bellerica. Our findings suggest that, an aqueous extract of *Triphalachurna*, traditional Ayurvedic herbal medicine is a potent antibacterial agent against common bacterial pathogens that infect various types of wounds including burns. It can be used in the treatment of wide range of infectious diseases. The results obtained in this study justify the use of *Triphalachurna* in majority of Indian population in treating microbial diseases.

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