Research Article

In vitro Anti-Microbial Activities of Triphala

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ABSTRACT

Triphala (tri = three and phala = fruits), is an ayurvedic preparation composed of three equal proportions of herbal fruits native to the Indian subcontinent: viz. *Terminalia chebula*, *Emblica officinalis* and *Terminalia belerica*. Triphala is a tridoshic rasayana having a balancing and rejuvenating effect on the three constitutional elements that govern the human life. Triphala is rich in anti- oxidants, possess antibacterial, anti-viral and anti-cancer properties. Triphala is rich in polyphenols, Vitamin C and flavonoids. The present study intends to provide an overview of the antimicrobial activities of the crude extracts of commercially purchased dried powdered Triphala with special emphasis on their pharmacological actions. Triphala acetone extract with DMSO was assayed *in vitro* by agar cup method against two clinical gram negative isolates of bacteria such as *Escherichia coli* and *Klebsiella pneumonia* and two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger*. The acetone extracts of Triphala exhibited slightly higher antifungal activity than antibacterial activity. The extract exhibited more inhibition against *E. coli* and *A. niger*. On the basis of the results obtained, it may be concluded that this polyherbal formulation are rich in phytochemical constituents exhibiting anti- microbial properties.

Keywords: Triphala, *In vitro*, Anti-microbial, Anti-bacterial activity, Anti-fungal activity.

INTRODUCTION

In ayurveda, triphala is a well-known poly herbal formulation. In Indian system of medicine (ISM), it is considered to be a rasayana drug[1]. Triphala is a mixture of the dried powders of three fruits namely *Emblica officinalis* Garetn (Euphorbiaceae), *Terminalia belerica* Linn (Combretaceae) and *Terminalia chebula* (Combretaceae) in equal proportions. Triphala is one among the ayurvedic medicinal herbal formulation mostly preferred by medical practitioners[2]. It can be used by all people irrespective of their age. In ayurveda it is described as a tridoshic rasayana that can balance and rejuvenate the three constitutional elements that govern human life i.e.; vata, pitta and kapha. It has various applications in medical field like laxative, eye rejuvenator and so on. It is very effective in headache, dyspepsia, ascites, leucorrhoea, also used as a blood purifier and possess anti- inflammatory, analgesic, anti-arthritic, hypoglycaemic and anti-aging properties. Triphala is claimed to have anti- viral and anti- bacterial effects[3]. Triphala is prescribed for fatigue, assimilation, reduces oxidative stress and infectious diseases such as tuberculosis, pneumonia, AIDS, periodontal diseases etc.[4] Triphala is reported to reduce considerably the damage due to oxidative stress[5]. Studies show that it inhibits the growth of Gram-positive and Gram-negative bacteria[6]. The recent studies prove that triphala is rich in gallic acid, vitamin C, ellagic acid, chebulic acid, bellaricanin, beta – sitosterol and flavonoids[7].

The aqueous and ethanol extracts of triphala and its individual components were found to have anti-bacterial activities against *Pseudomonas aeruginosa, Klebsiella pneumoniae, Shigella sonnei, Shigella flexneri, Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi-B, Escherichia coli, Enterococcus faecalis and Salmonella typhi* isolated from human immuno deficiency virus (HIV) infected patients[8]. Triphala controls dental plaque, gingival inflammation and microbial growth caused by *Streptococcus mutans* and *Lactobacillus*. Triphala controls plaque from baseline and its activity is comparable to commonly available mouthwash Chlorhexidine[9].

MATERIALS AND METHODS

Preparation of Plant Extract for Anti-microbial Screening

The Ayurvedic drug selected for the present study is Triphala**.** Triphala powder was purchased from local Arya Vaidyasala Kottaykkal chirayinkeezhu.

The dried powdered sample was then extracted with acetone and subjected to mechanical shaking at 100 rpm for 48 hours at room temperature. Supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume.

For anti-microbial screening the concentrated, dried powdered extract was dissolved in 10% dimethyl sulfoxide (DMSO) and were stored at 4°C for further use.

Test Organisms: - Anti- bacterial activity was evaluated against two selected gram negative pathogens such as *Escherichia coli* and *Klebsiella pneumonia* whereas anti-fungal against two clinical fungal isolates such as *Candida albicans* and *Aspergillus niger* (as recommended by the National Committee for Clinical Laboratories Standards, NCCLS), purchased from Biogenix Research Centre, Valiyavila, Thiruvananthapuram. In order to access the biological significance and ability of the plant part, the

minimal inhibitory activity was determined by Agar Cup Plate Assay Method.[10]

Anti-bacterial activity: - Petri plates containing 20ml of Muller Hinton medium were seeded each with 24hrs old culture of bacterial strains such as *E. coli* and *K. pneumonia* (growth of culture adjusted according to McFards Standard, 0.5%). Wells of approximately 10mm diameter were bored using a well cutter and 25µl, 50µl and 100µl of the extracts were added to the wells from a stock concentration of 0.1g/1ml. The plates were then incubated at 37°C for 24 hours. Anti-bacterial activity was assayed by measuring the diameter of the inhibition zone in millimetres formed around the wells. Streptomycin (standard anti-bacterial agent, concentration: 20mg / ml) was used as the positive control.

Anti-fungal activity: - Anti-fungal activity was also determined by Agar Cup Method. Potato Dextrose agar plates were prepared and overnight grown cultures of fungi such as *C. albicans* and *A. niger* were swabbed. Wells of approximately 10mm diameter were bored using a well cutter and extracts of 25 µl, 50 µl and 100 µl concentrations were added and the zones of inhibition were measured after overnight incubation which were then compared with that of standard antibiotics. Clotrimazole (standard antimycotic agent, concentration: 10mg / ml) was used as the positive control.

RESULTS AND DISCUSSION

Anti-Bacterial Activity

The anti-bacterial activity of triphala extract (Acetone extract with DMSO) was assayed *in vitro* by agar cup method against two clinical gram negative isolates of *E. coli* and *K. pneumonia*. Standard antibiotics were tested for their activity and their zones of inhibition were recorded. Among the varying concentration of triphala extracts both bacteria had positive results. *E. coli* exhibited maximum activity whereas *K. pneumonia* exhibited slightly lesser activity at higher concentrations.

The sequence of anti-bacterial activity of triphala extract against *E.coli* exhibited maximum activities of 11, 12, 17 mms of inhibition zones respectively at all the three different concentrations (25µl, 50µl, 100µl). Whereas the sequence of anti-bacterial activity against *K. pneumonia* exhibited 11, 13 and 15 mms of inhibition zones at 25, 50 and 100µl concentrations. (Table 1)

Table 1: Zone Diameter of Inhibition of Acetone Extract of Triphala

Test Organisms	Zone of Inhibition in mm			Positive Control
	Concentration of Triphala Extracts (µl)			
	25	50	100	
E. coli	11	12	17	41
K. pneumonia	11	13	15	41

Note: Concentration of Sample Stock: 1mg/ml DMSO

Anti-bacterial activity of the acetone extract of triphala against *E. coli* and *K. pneumonia*.

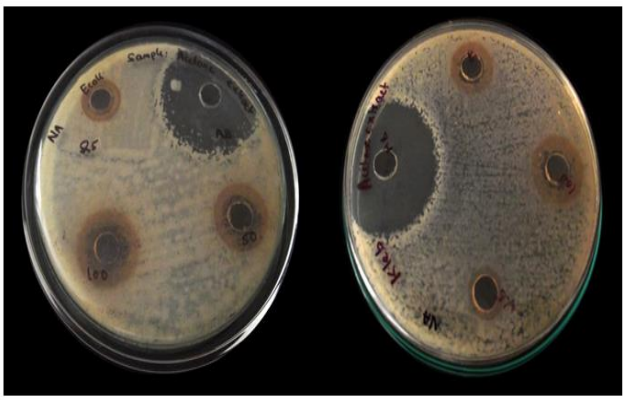

Plate 1 Plate 2

Plates 1 & 2 showing the zone of inhibition produced by the acetone extracts of triphala at 25µl, 50µl & 100µl concentrations against ***E. coli*** and *K. pneumonia*.

Anti-Fungal Activity

The anti- fungal activity of triphala extract (Acetone extract with DMSO) was assayed *in vitro* by agar cup method against two clinical fungal isolates such as *C. albicans* and *A.niger*.

Table 2 shows the zone of inhibition produced by the extracts on Muller Hinton agar against the respective fungal strains.

Triphala showed ostensible anti-fungal property with *Aspergillus niger* exhibiting maximum activity and *Candida albicans* exhibiting lesser activity at high concentrations.

The sequence of anti-fungal activity of triphala extracts against *C. albicans* produced inhibition zones of 10, 13 and 17mms in 25, 50 and 100µl concentrations respectively.

While inhibition zone of 11, 14 and 18mms were produced against 25, 50 and 100µl concentrations on *A. niger*.

The present study reveals that the acetone powder extracts of triphala were active against both fungal strains viz. *C. albicans* and *A. niger*. But it gave better results against *A. niger*.

Table 2: Zone Diameter of Inhibition of Acetone Extract of Triphala

Test Organisms	Zone of inhibition in mm			Positive Control
	Concentration of Leaf Extracts (µl)			
	25	50	100	
C. albicans	10	13	17	31
A.niger	11	14	18	33

Concentration of Sample Stock: 1mg/ml DMSO

Anti-fungal Activity of the Acetone Extract of Triphala against *C. albicans* and *A. niger.*

Plate 3 Plate 4

Plates 3 & 4 showing the zone of inhibition produced by the acetone extracts of triphala at 25µl, 50µl & 100µl concentrations against *C. albicans* and *A. niger***.**

CONCLUSION

Triphala (tri = three and phala = fruits), include the Arabicized "Atrifal" and the Chinese term "San-Teng". This preparation is composed of three equal proportions of herbal fruits: *Terminalia chebula*, *Emblica officinalis* and *Terminalia belerica*. Triphala is a tridoshic rasayana having a balancing and rejuvenating effect on the three constitutional elements that govern the human life. Triphala is rich in antioxidants, possessing antibacterial, anti-viral and anti-cancer properties. Triphala is also known to cure cataract and effective in treatment of Acquired immune deficiency syndrome (AIDS).

Triphala is rich in many polyphenols, Vitamin C and flavonoids. Triphala is a polyherbal formulation and the mechanism of action of polyherbals/herbal drugs and their extracts differ in many respects from that of the synthetic drugs or single substances. In ayurvedic terms, triphala, used in moderation, is said to have a beneficial effect on all three doshas—vata, pitta and kapha. It is most well-known for its gentle effects on the bowels, improving peristalsis and cleansing toxic build-up of wastes; but ayurveda also views triphala as a nourishing supplement known for its ability to rejuvenate healthy tissues, allowing one to age gracefully and that is why triphala is also termed as 'nectar of life. This obtained information will therefore serve as a primary platform for further phytochemical and pharmacological studies of Triphala. Hence it can be concluded that this herbal formula would direct to the establishment of some compounds that could be used to invent new and more potent anti-microbial drugs of natural origin. Therefore, future research should be addressed on the application of using the aforesaid ayurvedic medicine as natural remedied and to protect against infectious diseases.

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