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Study of antimicrobial activity of Triphala and its individual components

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Abstract
Drug resistance has developed due to indiscriminate use of existing allopathic antimicrobial drugs and become a global health problem. This resistance problem demands renewed efforts to seek antimicrobial agents from other sources. Herbal Products of higher plants has antimicrobial compounds with possibly novel mechanism of action with minimum side effects. Triphala is a herbal formulation, consisting of equal parts of fruits of Amla (*Emblica officinalis*), Harde (*Terminalia chebula*) and Baheda (*Terminalia bellerica*). These are claimed to have antiviral, antifungal and antibacterial effects. Present study was aimed to identify antimicrobial effect of Triphala and its individual components against common bacterial isolates, *Candida* and to compare the antimicrobial effect of water and DMSO based extracts of it. Triphala and its components were dissolved in distilled water and also in DMSO to get a final concentration of 100 mg/ml. Suspensions of *S. aureus*, Coagulase Negative Staphylococci, *Enterococci sp*., *Lactobacillus sp*., *E. coli*, *Klebsiella sp*., *Pseudomonas sp*., *Candida sp*. were inoculated on Mueller Hinton agar (MHA). Wells were made on MHA plates and herbal extract (100 µl) were added in to it. After 18 hours of incubation at 35 °C, zone of inhibition surrounding wells were measured and recorded. We have observed antimicrobial activity of each component against most of organisms. It was also observed that triphala is having more antimicrobial effect compared to its individual components. We had not observed significant difference in antimicrobial effect between water and DMSO extracts of these herbal preparations.

Keywords: Triphala, amla, Harde, Baheda, antimicrobial effect

1. Introduction
In recent years, drug resistance has been developed due to indiscriminate use of existing allopathic antimicrobial drugs in the treatment of infectious diseases and has become a global public health problem [1]. This resistance problem demands a renewed effort to seek antimicrobial agents from other sources effective against pathogens [2]. Herbal Products of higher plants has antimicrobial compounds with possibly novel mechanism of action [3, 4]. That are effective for treatment of infectious diseases and also not having side effects that often associated with conventional antibiotics [5].

Triphala is an herbal formulation, consisting of equal parts of fruits of three medicinal plants namely, Amla - *Emblica officinalis* Gaertn (Family: Euphorbiaceae), Harde - Terminalia chebula Retz (Family: Combretaceae) and Baheda - *Terminalia belerica* (Gaertner) Roxb (Family: Combretaceae). Triphala is traditionally prescribed in ayurveda for various diseases since antiquity. The fruit components of Triphala are claimed to have antiviral, antifungal and antibacterial effects [6].

Present study has been designed to evaluate antimicrobial potential of Triphala as a whole and its individual components against common pathogens.

2. Aims and Objectives
- To study anti-bacterial effect of Triphala and its individual components against common bacterial isolates.
- To study anti-fungal effect of Triphala and its individual components against *Candida sp*.
- To compare the antimicrobial effects of water based and DMSO based extracts of Triphala and its individual components.

3. Materials and Methods

3.1. Study of antimicrobial activity of herbal preparation

This study of Triphala and its individual components Amla, Harde and Baheda was conducted in Microbiology Laboratory at C. U. Shah Medical College, Surendranagar (Gujarat) India.

3.2. Herbal compound preparation

3.2.1. Base Water

Herbal preparations: Triphala and its individual components Amla, Harde and baheda were obtained in powder form from Lions brand ayurvedic pharmacy. All four herbal preparations were suspended in distilled water to get a final concentration of 100 mg/ml.

3.2.2. Base DMSO

Simultaneously same above four herbals powder were suspended in 0.5% DMSO (Dimethyl sulfoxide – an alcohol based solvent) to get a final concentration of 100 mg/ml [7].

3.2.3. Media preparation for test

Mueller Hinton agar (MHA) was obtained from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. MHA was prepared and sterilized by autoclaving and poured into sterile 10 cm diameter petri plates up to 4 mm agar thickness in every plate.

3.2.4. Microorganism Suspension

Table 1: List of Microorganism tested

Gram positive bacteria	*S. aureus*, CoNS (Coagulase Negative Staphylococci) *Enterococci sp.* *Lactobacillus sp.*
Gram negative bacteria	*E. coli* *Klebsiella sp.* *Pseudomonas sp.*
Fungus	*Candida sp.*

These organisms were obtained from clinical specimen. They were isolated in pure culture and identification was done by biochemical reaction as per standard methods [8]. To test antimicrobial property of herbal preparation, suspensions of organisms were prepared in peptone water and turbidity were adjusted to 0.5 McFarland standard for bacteria and 2.0 McFarland standard for *Candida* as per the Clinical and Laboratory Standards Institute (CLSI) guidelines [9].

3.2.5 Test

Suspensions were inoculated on surface of MHA plates by lawn culture method using sterile swab and allowed to dry. Wells were made measuring 6mm in diameter on inoculated MHA plates using standard borer from Hi-Media Laboratories Pvt. Ltd. Mumbai, India. Herbal extract (100 µl) were added in different wells using calibrated micropipette. All inoculated plates were incubated aerobically at 35 ˚C for 18 hours in laboratory incubator. Zone of inhibition surrounding wells were measured and recorded [7].

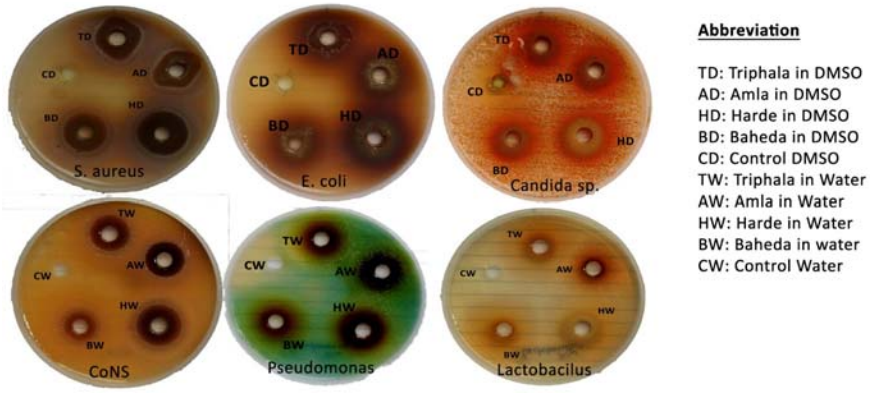

Image 1: Zone of Inhibition for various microorganism

4. Result

Zone of inhibition surrounding wells were shown in Table 2 and 3 for water based and DMSO based preparation respectively, that represent anti-microbial activity of respective herbs.

Table 2: Antimicrobial activity of Triphala and its component (zone of inhibition size in mm) - Solvent Water

Organism / Compound	*S. aureus*	CoNS	*Klebsiella*	*Pseudomonas*	*E. coli*	*Candida*	*Enterococcus*	Lactobacilli
TRIPHALA	22	19	0	24	13	12	14	23
AMLA	21	20	0	17	14	0	16	20
HARDE	26	24	0	24	11	11	18	28
BAHEDA	18	14	0	22	0	16	12	22

Table 3: Antimicrobial activity of Triphala and its component (zone of inhibition size in mm) - Solvent DMSO

Organism / Compound	*S. aureus*	CoNS	*Klebsiella*	*Pseudomonas*	*E. coli*	*Candida*	*Enterococcus*	Lactobacilli
TRIPHALA	26	20	0	20	18	20	19	18
AMLA	24	18	0	19	0	21	19	20
HARDE	26	22	0	25	0	20	24	24
BAHEDA	22	18	0	21	0	21	20	19

We have observed antimicrobial activity of each component against all organisms except *Klebsiella sp*. It was also observed that triphala is having more antimicrobial effect compared to its individual components. Anti-bacterial activity was not demonstrated against *E. coli* by individual components in water but Triphala had shown significant effect. Amla has no antifungal effect against *Candida sp*. but Triphala had shown antifungal effect.

We had not observed significant difference in antimicrobial effect between water and DMSO extracts of these herbal preparations.

5. Discussion

The continuous emergence of multidrug-resistant (MDR) bacteria and high cost of production of synthetic antibiotics have necessitated the development of alternative antimicrobial agents from other sources [10]. Medicinal plants could be one of those alternatives because most of them are safe, cheap and affect a wide range of antibiotic resistant microorganisms. The rich chemical diversity in plants promises to be a potential source of antibiotic resistant modifying or modulating compounds and has yet to be adequately explored [11, 12].

Srikumar *et al.* [6] have reported the antibacterial potential of Triphala and its components against some drug-sensitive clinical isolates obtained from HIV infected patients. Rani P *et al.* [13] have reported activity of Triphala against multidrug-resistant Salmonella typhi. Anwesa B *et al.* [7] have also observed antibacterial potential of Triphala components against multidrug – resistant uropathogens. Jyotsna S *et al.* [14] have found that Triphala is equally effective in reduction of oral microbial flora as chlorhexidine. Another study done by Yogesh S *et al.* [15] in Pune Maharashtra have also shown growth inhibition of all Gram - positive and Gram – negative bacteria. We have also found very potent antimicrobial activity against common clinical isolates with Triphala and its individual components. However, the mechanism of action of these herbal agents on microbes is yet to be understood.

6. Conclusion

- Triphala and its individual components have significant anti-bacterial activity.
- Triphala and its individual components have significant anti-fungal activity against *Candida sp*.
- There is no significant difference in the antimicrobial effect of water based and DMSO based Triphala and its individual components.

7. Recommendation

- Like Triphala, other herbs can also be tested for their antimicrobial effects.
- It is necessary to develop universal reference methods for testing of herbs for their antimicrobial effects.

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