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In-vitro Antimicrobial Evaluation of Triphala Kwatha (Decoction) and its Alcoholic Extract on Wound Microbes

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Abstract

Triphala is the sanskrit term used in the traditional Indian system of medicine consisting of a combination of three herbal fruits namely *Hareethaki* (Terminalia chebula), *Vibithaki* (Terminalia bellerica), and *Amalaki* (Emblica officinalis). *Triphala kwatha*(Decoction) is a unique combination which has been used in Ayurveda for wound management since time immemorial. The study aimed to explore the possibilities of *Triphala kwatha* (decoction) and its alcoholic extract as an antimicrobial agent against wound microbes. In-vitro studies using Amox and Gentamycin as standards were done against four microorganisms i.e Staphylococcus aureus, Citrobacter, Escherichia coli, and Proteus species. These microorganisms were isolated from patient wound samples and identified by gram staining and biochemical reactions. The Agar well diffusion method or hole plate method and Agar disc diffusion method were used for In Vitro study. The in-vitro results found that *Triphala kwatha* (decoction) had more antimicrobial activity against microbes than the alcoholic extract of *Triphala kwatha choorna*. Also, it was evident that the activity of *Triphala kwatha*(decoction*)* was more against gram +ve organisms like Staphylococcus aureus.

Introduction

Triphala is a highly efficacious Ayurvedic herbal medicine and is made up of the deseeded fruits of *Harithaki, Vibithaki,* and *Amalaki*[1]. Varying classical textbooks in Ayurveda recommend different ratios for the *Triphala*'s distinct components. In Ayurveda, there are numerous formulations containing Triphala as the primary ingredient, including *Triphala choorna, Triphala kwatha, Triphala grutham, Triphala mashi, Triphala Rasayana, Panchagavya grutha, Mahapanchagavya grutha* and many more. Traditional Ayurvedic textbooks recommend *Triphala* for a variety of illnesses, including *Prameha, Vrana, Thimira, Vathavyadi,Visarpa, Sopha, Mukharoga,Nadivrana* and as *Rasayana. Triphala* is likely most known for its usage in *Vrana kshalana, Rasayana* and general gastrointestinal health promotion. Major traditional texts like the *Charaka Samhitha, Susrutha Samhitha,* and *Ashtanga Hrudaya*

cite *Triphala* as a significant medication for *Vrana chikitsa* (Ulcer management*)*. Since ancient times, *Triphala kwatha* (decoction*)* has been utilised for *Vrana kshalana*(wound cleaning). The ratio chosen for the current study *Harithaki: Vibithaki: Amalaki* in the ratio 1:1:1 was by the famous classical Ayurvedic textbook *Bhaishajya Ratnavali Upadamsika Vrana chikitsa* and also according to AFI[2]. For *Triphala*'s antimicrobial activity, numerous research has already been conducted, the majority of which used alcoholic extracts of *Triphala choorna*. Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecalis, and Acinetobacter baumanni are the most often encountered microbial species that cause wound infections[3]. But for the present study microorganisms were collected directly from the patient's wound sample. The isolated four microbes from the patient's wound samples were: Staphylococcus aureus, Citrobacter, Escherichia coli, and Proteus species.

One of the biggest problems in treating infectious diseases is the microbial resistance brought on by the overuse of antibiotics. Utilizing natural antimicrobial agents is a novel way to minimise this. Although hundreds of plant species have been tested for their antimicrobial properties, a vast majority of them remain unexplored. The present study aims to throw light on the antimicrobial effects of *Triphala kwatha* and its alcoholic extract, a stepping stone for exploring further studies on *Triphala Kwatha*.

Materials And Methods

Collection of raw materials:

Drugs Terminalia chebula, Terminalia bellerica and Emblica officinalis were collected from genuine sources and authenticated from the Pharmacognosy department.

Preparation of medicine:

Deseeded fruits of each drug in the combination of *Triphala* (Terminalia chebula, Terminalia bellerica and Emblica officinalis) were collected separately, washed thoroughly, removed all earthy and foreign matter and dried in shade. Then the drugs were subjected to pulverization and made into a coarse powder.

Two forms of *Triphala* were used.

1. *Triphala Kwatha* (Decoction)

Kwatha was prepared by taking *Hareethaki, Amalaki, and Vibitaki* in equal quantities (1:1:), took 1pala of the drug (48g), adding 16 times oater (768ml) to it, and then it was boiled and reduced into half the initial quantity (384ml)[4]. The ratio of *Hareethaki, Vibithaki and Amalaki* were selected according to the Ayurvedic Formulary of India.[2] And the method of *kwatha* (decoction) prepared was according to *Haareetha Samhita*[9]

2. Alcohol Extract of *Triphala kwatha choorna*

250 g of the powdered drug were weighed and put in a round bottom flask and was reflexed with 500 ml of Ethyl alcohol using a Lebig's condenser for 2 hrs. The extract obtained was distilled and evaporated to dryness.

Isolation of microorganisms from clinical samples: Microorganisms were collected directly from patient samples by taking wound swabs. Characterisation of organisms done by gram staining.

Gram-negative organisms: Citro bacter, Proteus mirabilis, Escherichia coli.

Gram positive organism : Staphylococcus aureus,

Standard drugs used: Gentamycin10µg and Ampicillin 10 µg

Method Of Identification Of The Organisms

a) Gram staining

Escherichia coli, Proteus species, and Citrobacter were found to be Gram-negative. While Staphylococcus aureus is Gram-positive.

b) Biochemical reactions

i. Catalase test

Staphylococcus aureus and Proteus species produced bubbles so they were catalase positive.

ii. Coagulase test

Clumbing on rabbit plasma occurred in the case of Staphylococcus aureus which shows a positive test.

iii. Oxidase test

Among the three Gram-negative organisms , no one showed a positive result.

iv. Oxidative fermentative test

E.coli are oxidative and fermentative in the result. Proteus species and Staphylococcus aureus were fermentative but not oxidative.

v. Nitrate reduction test

All four tested organisms showed positive results on this test.

vi. Sugar fermentative test

E.coli and Staphylococcus fermented all the sugars, the first organism produce both acid and

gas while Staphylococcus produced only acid.

vii IMViC test

E.coli was found to be indole positive while the others are indole negative. E.coli, Proteus species, and staphylococcus were positive for methyl red. E.coli and staphylococcus aureus were found to be negative for citrate.

viii. Urease test

Except for E.coli, all the other organisms were found to be positive for urease.

ix. Triple sugar iron agar test

E.coli produced both acid slant and acid butt with gas production (indicates lactase and sucrose fermentation). Acid slant and acid butt without any gas production occurred in case the of S. aureus. Proteus produced an alkaline slant with blackening in the butt (indicates H2S production without carbohydrate fermentation)

Method Of Invitro Antimicrobial Study

Invitro antimicrobial study was carried out in Drug Standardization Unit, Government Ayurveda College, Thiruvananthapuram. Four microorganisms viz Citrobacter, Proteus species, E.coli and Staphylococcus aureus were collected from patient wound samples tested for antimicrobial activity.

Table 1 :BIOCHEMICAL REACTIONS OF GRAM-NEGATIVE TEST ORGANISMS

Test organism	Catalase	Oxidase	Oxidative fermentative	Nitrate Reduction	Sugar fermentation			D"i.c				Urease	TSI
					Glucose	Lactose	Sucrose	Indole	MR	VP	Citrate		
Escherichia coli	+	-	Fermentative	+	AG	AG	AG	+	+				A/A)
Proteus mirabilis	+	-	Fermentative	+	AG			D	+	-	D	+	A, H2S
Citrobacter	+	+	Oxidative	+	A	-		-	-	-	+	+	K/K

[AG-Acid gas production, A / (A)-Acid slant, Acid butt and gas production, K /A, H_2S-Alkaline slant, Acid butt with H_2S production, MR-Methyl Red test, VP-Voges Proskauer test, D-Variable]

The two methods adopted for the In-vitro study are Agar well diffusion method and the Agar disc diffusion method

1.The Agar well diffusion method or hole plate method [5]

This method was used for screening the antimicrobial action of *Triphala Kwatha*(Decoction) and Alcohol Extract of *Triphala kwatha choorna*. The media used for this method was Muller Hinton Agar (MHA). MHA media was prepared and autoclaved at 1210 C 15 lbsMts mts. The medium was poured into sterile Petri dishes and allowed to solidify. Cut wells on the plate with a well puncture and wells are marked for each dilution of extract. Sterilized peptone broth was inoculated with 5 test organisms and incubated for 2 – 4 hrs. at 3 – 70 C. Lawn culture of the test organism was made on the MHA plates by using sterile cotton swabs. 50 µd of different dilutions of each extract were pipetted into the desired wells in each plate. Zone of inhibition was measured by using a ruler and marked in millimetres.

2. Agar disc diffusion method

This method was used for testing antibiotic susceptibility. Antibiotic discs, Gentamycin and Ampicillin/cloxacillin of concentration 10mcg / disc were aseptically put on the sterilized MHA plates lawn cultured with each test organism. Zone of inhibition was measured using a ruler and marked in millimetres.

Result

Isolated microbial cultures undergo various biochemical reactions and the results are given in table 1 & table 2

The zone of inhibition obtained for *Triphala Kwatha* (Decoction) and Alcohol Extract of *Triphala kwatha choorna* and the standard drug has been summarized in Table 3 and Table 4 respectively and the images of the culture of organism depicting the zone of inhibition are given in Figure1.

Table 2:BIOCHEMICAL REACTIONS OF GRAM-POSITIVE TEST ORGANISM

Test organism	Catalase	Oxidative fermentative	Nitrate Reduction	Sugar fermentation			H " ic				Urease
				Glucose	Lactose	Sucrose	Indole	MR	VP	Citrate	
Staphylococcus	+	+	Fermentative	+	A	A		+	+		+

[AG-Acid gas production, A / (A)-Acid slant, Acid butt and gas production, K /A, H_2S-Alkaline slant, Acid butt with H_2S production, MR-Methyl Red test, VP-Voges Proskauer test, D-Variable]

Table 3: Action of *Triphala kwatha* on microbes

No:	Name of the microorganism	Zone of inhibition (mm)	Antibiotics used Gentamycin 10	 Ampicillin 10
1	Citrobacter	17	17	-
2	Proteus species	15	20	-
3	E.coli	19	30	-
4	S.aureus	20	-	10

Table 4: Action of Alcohol extract of *Triphala kwatha choorna* on microbes

No:	Name of the microorganism	Zone of inhibition (mm)	Antibiotics used Gentamycin 10	 Ampicillin 10
1	Citrobacter	11	17	-
2	Proteus species	24	20	-
3	E.coli	28	30	-
4	S.aureus	17	-	10

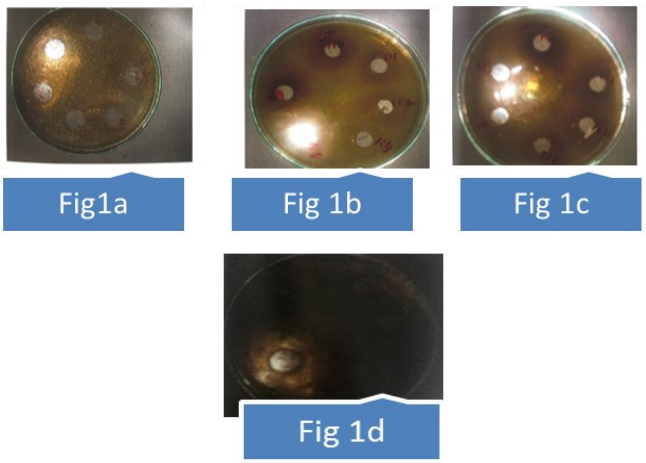

Figure 1: Cultures of an organism with Zone of inhibition against different concentration a. Staphylococcus aureus b.Citrobacter c.Proteus species d. E.coli

Discussion

Current advancements in drug discovery and the search for novel chemical diversity have intensified the efforts for exploring leads from "*Ayurveda*", the traditional system of medicine in India. Although extremely effective, antibiotics are prone to induce resistance in bacteria. For more than 50 years, bacterial resistance has been the main factor responsible for the increase in morbidity, mortality and healthcare costs of bacterial infections. *Triphala* is used in Ayurveda medicine in treating a variety of conditions and also forms part of many other Ayurveda formulations.

After the review of the literature, the ratio 1:1:1 was sorted out for the individual components in *Triphala* and the said ratio was found to be effective in the management of wounds (*Vrana chikitsa*) when used externally.

Various dosage forms of *Triphala* find extensive use in classical literature. Among them, *kwatha* (decoction) was selected on account of its specific use in the management of wounds (Vrana)[6]. Different methods of preparation of *kwatha* are available in classics. The word *Kshalana kwatha* (decoction for washing) was first mentioned in *Bhoja Samhitha*. For *Hareetha Samhita* was opted for and reviewed for the preparation of *Kshalana kwatha* (decoction for washing). As discussed in the literature part

among *sapta vidha upakramas Triphala kwtaha* is indicated both in '*sodhana* (cleansing) and *ropana (*healing)' upakramas. Considering the rasas of individual drugs *Hareethaki* and *Amalaki* possess all rasas except *lavana* (salt). *Vibithaki* is said to have *kashaya thiktha rasa. Tiktha rasa*, itself is having properties of *vrana sodhana , vrana ropana and kledha soshana*. . The drug *Vibithak*i is said to have '*Krimi nasaka' karma*, which might point towards the antimicrobial action of *Triphala.*

Triphala Churna's antibacterial effects against human diseases were discovered by in silico testing against microorganisms. Triphala churna chemicals follow Lipinski's rule of five, according to in silico data[7]. An In-vitro study was carried out for proof of the antimicrobial activity of *Triphala kwatha*. Both *Triphala kwatha* and the alcohol extract of *Triphala kwatha choorna* were taken for the study. The therapeutic efficacy of the formulation on the growth of four microorganisms viz Citrobacter, Proteus species, E.coli and S.aureus was assessed. All the microorganisms were identified by gram staining and through different biochemical reactions. Cultures of all tested organisms showed a clear Zone of inhibition and the antibiotics used for the study were Gentamycin and Amox. *Triphala* possesses more action against microbes compared to antibiotic disc used. From the in-vitro results, it was found that *Triphala kwatha* is having more antimicrobial activity against microbes than the alcoholic extract of *Triphala kwatha choorna*. *Kwatha* is a pure water extract, thermostable and filtered through a fine cloth. The *Kwatha* constitutes organic compounds, soluble fibres, inorganic matter and trace elements. So the activity is threefold when compared to the alcohol extract of *Triphala*. Also, it was evident that the activity of *Triphala kwatha* is more against gram +ve organisms like S.aureus. Since gram +ve organisms is having a single cell membrane while gram –ve organisms possess multiple cell membranes.

Triphala contains Tannin and Gallic acid as the major chemical constituents. These two might be the factors accounting for the therapeutic efficacy of *Triphala kwatha*. The antimicrobial mechanisms of tannins are well-known [8]. Its astringent property can induce complexing with enzymes or substrates. Many microbial enzymes in raw culture filtrates or purified forms are inhibited when mixed with tannins. This Tannin - toxicity may be related to its action on the membranes of microorganisms. The complexation of metal ions by tannins may be the reason for tannin- toxicity. The ester linkage between gallic acid and glucose contributes to the antimicrobial potential of these compounds. These Tannins and Gallic acids are water-soluble plant molecules. So their action will be high when used as water extracts. That is the reason behind the increased activity of *Triphala kwatha* than its alcohol extract against microbes.

Conclusion

From the above results, it is evident that *Triphala kwatha (*decoction) is enriched with the property of reducing the growth rate of microorganisms in a wound. *Triphala kwatha* (decoction) is proven to be more potent than the alcohol extract of *Triphala kwatha choorna* in reducing microbial growth present in a wound. The antimicrobial activity of *Triphala kwatha*(decoction) is undisputed, especially against Gram +ve Bacteria like Staphylococcus aureus. Hence scientific evidence supports Triphala kwatha(decoction) as a very promising treatment option for wound cleaning and healing.

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