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ORIGINAL RESEARCH ARTICLE

Antimicrobial Activity of *Ayurvedic* Hand Sanitizers

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ABSTRACT

Hand hygiene is the best tool in preventing opportunistic infections caused by different micro organisms and to achieve this, the use of hand sanitizers becomes must in current scenario. The purpose of present study was to prepare an *Ayurvedic* hand sanitizers incorporating *Panchavalkala*, the known herbal combination with multi dimensional activities and to evaluate their respective antimicrobial activities. The results were insignificant with antiseptic liquid, but Gel hand wash showed encouraging results in culture sensitivity.

Keywords: *Ayurveda*, Hand sanitizer, *Panchavalkala*, antimicrobial activity

INTRODUCTION

Ayurveda, the first organized system of medicine, ever evolved throughout the globe, is not mere a system dealing with healing techniques and curing diseases. It is indeed a codified science which issues definite guidelines for healthy, peaceful and happy living and maintenance and protection of physical and psychological health, with an object of achieving longevity. The system has been primarily developed with two basic objectives viz. maintenance of health and prevention of disease in a healthy individual and eradication of diseases in diseased. The first objective is achieved by observance of guidelines related to healthy living and the second objective specifically deals with disease curative aspect. Thus it is very clear that *Ayurveda* is not only a curative medical science but also is a comprehensive way of healthy living.

Guidelines related to healthy or '*swastha*' individual are termed as '*Swastha vritta*'[2] and regular practices are considered in relation to daily practices known as '*Dinacaryaa*' in Ayurveda.

Cleanliness of the body carries utmost importance in maintenance of health. In this respect it has been emphasized that cavities of ear, nose and crevices of foot and hands should be cleaned regularly and no dirt should be allowed to accumulate in these places. Maintaining physical cleanliness and use of de-odorants are essentials of healthy living [3]. These concepts highlight the need of maintaining hygiene in prevention of diseases.

Though proper hygiene and in particular hand hygiene is the single most important, simplest, and least expensive means of preventing health care-associated infections and the spread of antimicrobial resistance; but, unfortunately poor hand-hygiene practices are still observed due to lack of scientific knowledge, unawareness of risks and unavailability of hand-hygiene facilities[4,5,6].

In the current scenario of mechanized life style; a consumer will always prefer ready-made dosage forms. Considering this demand; an attempt has been made to screen classical literature for the herbs with cleansing properties and found that, *Pancha Valkala*[7] (the barks of five plants) has activities like *vranaprakshalana*[8], *vranaropana*[9], *shothahara*[10], *upadanshahara*[11] *visarpahara*[12].

Pancha Valkala is the combination of barks of five different plants viz. *Vata* (*Ficus bengalensis* Linn.), *Udumbara* (*Ficus glomerata* Roxb.), *Ashwattha* (*Ficus religiosa* Linn.), *Parisha* (*Thespesia populnea* Soland. ex Correa.), *Plaksha* (*Ficus lecor* Buch. Ham.).

Kwatha (aqueous extract) of *Pancha Valkala* was formulated in to anti septic liquid and gel by using suitable excipients; which can be used as a ready-made herbal hand wash.

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MATERIALS AND METHODS

Raw Material: Barks of *Panchavalkala* were procured from Pharmacy, Gujarat Ayurved University, Jamnagar. Macroscopy and powder microscopy of the five drugs were done in order to evaluate the genuinity and authenticated by Dept. of Pharmacognosy, IPGT & RA, and GAU.

Formulations [13]**:** *Panchavalkala kwatha* (**Table 1**) was prepared by following specified classical guidelines. The prepared *Kwatha* was used as the base in preparing antiseptic liquid and gel hand wash in the department of *Rasashastra* and *Bhaishajya Kalpana*, IPGT & RA, GAU.

METHOD OF PREPARATION:

***Kwatha*:** Coarse powder (sieve No. 10) of the barks of *Panchavalkala* was prepared and soaked in 16 times of water for overnight. Next day it was subjected to heat with continuous stirring and the quantity was reduced to 1/4th of the initial volume. The liquid was filtered through four folded clean cotton cloth and the filtrate was collected as *Panchavalkala Kwatha*. (**Table 1**)

Antiseptic Liquid [14]**:** 35% of *Panchavalkala Kwatha*, 56% of Isopropyl Alcohol and 7% of Acetone were mixed in a stainless steel container. 2% glycerin was added to above mixture and was homogenized with a stirrer. Prepared product was stored in air tight HDPE containers. (**Table 2**)

Gel Hand Wash [15]**:** 2% carbopol was added to 77.6% of *Panchavalkala Kwatha* with constant stirring in a stainless steel container placed in a water bath maintaining temperature in between 60 - 70^0. After uniform mixing; the combination was allowed to cool to 40 - 45^0 and 20% of Sodium Lauryl Ether Sulfate (SLES) and 0.2% of Tri Ethanol Amine (TEA) were added with slow stirring to avoid formation of possible air bubbles in the product. Finally, 0.1% each of Methyl & Propyl Paraben as a preservative and 0.5% of perfume and mixed with slow stirring to obtain uniform product. Prepared product was stored in air tight HDPE containers. (**Table 3**)

Table 1: Composition of *Panchavalkala Kwatha*:

SNo	Drugs	Latin Names	Part used	Proportion
1	*Vata*	*Ficus bengalensis* Linn.	Bark	1 Part
2	*Udumbara*	*Ficus glomerata* Roxb.	Bark	1 Part
3	*Ashwattha*	*Ficus religiosa* Linn.	Bark	1 Part
4	*Parisha*	*Thespesia populnea* Soland. ex Correa.	Bark	1 Part
5	*Plaksha*	*Ficus lecor* Buch. Ham.	Bark	1 Part
6	Water	--	--	16 Parts
		Reduced to 1/4th	--	4 Parts

Table 2: Formulation composition of antiseptic liquid:

SN	Ingredients & Excipients	Quantity Taken %	Quantity Taken g/ml
1	*Panchavalkala Kwatha*	35	350
2	Isopropyl Alcohol	56	560
3	Acetone	7	70
4	Glycerin	2	20

Table 3: Formulation composition of gel:

S.N	Ingredients & Excipients	Quantity Taken %	Quantity Taken g/ml
1	*Panchavalkala Kwatha*	77.6	776 ml
2	Carbopol	2	20 g
3	Sodium lauryl ether sulfate	20	200 g
4	Tri ethanol amine	0.2	2 ml
5	Methyl paraben & propyl paraben	0.1 / 0.1	1 g / 1 g
6	Perfume (berybery)	Q.S.	5 ml

ANTIMICROBIAL ACTIVITY:

In-vitro tests are used as screening procedure for new agent and for testing the susceptibility of individual isolates from infections to determine which of the available drug might useful therapeutically. In general any compounds or drugs which inhibit the growth or cause the death of micro organisms is known as antimicrobial agent. Any drug, which inhibits the growth of bacteria or fungi is said to possess bacteriostatic and fungistatic activity respectively.

The prepared antiseptic liquid and gel were evaluated for anti bacterial activity by culture sensitivity test.

Sensitivity Testing Method:

Agar Diffusion Method (Cup plate Method): In current study the antimicrobial activity of trial drugs were carried out by the agar diffusion method. Different concentrations of antiseptic liquid and gel were incorporated into an agar medium in a petridish. Replicator device was used to inoculate multiple specimens on to a series of plates with varying concentration of antibiotics and responses of organisms to the trial drugs were measured and compared with the response of the standard reference drug. Ampicillin was the standard reference for antibacterial study, whereas Amphoterecin - B for antifungal activity.

Micro organisms: Anti microbial activity of the trial drug was evaluated in the below specified organisms.

Bacteriae

1. Escherichia coli (gram –ve) ATCC 10531

2. Pseudomonas aeruginosa (gram –ve) ATCC 25619
3. Staphylococcus aureus (gram +ve) ATCC 6538
4. Bacillus pumillus (gram +ve) ATCC 14884

Fungi

1. Sacchuromyces cerevisiae (yeast) ATCC 2601
2. Candida albicans (yeast) ATCC 10231

Standard Inoculum: Standardization of the inoculums is essential to provide reproducible MICs (Minimum Inhibitory Concentration). It is determined by comparing the turbidity of the liquid medium to a standard that represents a known number of bacteria in suspension. Here inoculums prepared by comparing with Mc Farland standards.

Requirements:

1. Nutrient Agar (For bacterial cultivation).
2. PDA (Potato Dextrose Agar) for fungal cultivation.
3. Young Culture of micro organisms - 12-15 hrs (Overnight) incubated.
4. Standard solution antibiotics.
5. Different concentrations of trial drugs.
6. Sterile petridish and sterile cork borer.
7. Incubator and LAF (Laminar Air Flow Cabinet)

Procedure:

1. Stock solution of antibiotic concentration (1000mg/ml) was prepared.
2. Different (as mentioned in results) concentrations of trial drugs were prepared.
3. A medium suitable for the condition (Nutrient Agar for bacterial and PDA - Potato Dextrose Agar for fungal cultivation) of the assay was liquefied and inoculated at a suitable temperature e.g. 48 to 50˚C for ve getative forms, with a known quantity of a suspension of micro organ.
4. Immediately it was poured into the petridishes of the inoculated medium to form uniform layers of 2mm to 5mm thickness.
5. The plates were incubated at room temperature for one hour, added with the trial drugs of known concentrations in the well and then incubated in bacteriological incubators at 37˚C for 24 hours for bacteria and in B.O.D. incubator at 22˚C for 24 to 48 hours for fungi.
6. Well was prepared by sterile cork borer.
7. After incubation the zone was measured with the help of digital zone reader and compared with the standard drugs.
8. From zone of inhibition the MIC (Minimum Inhibitory Concentration) value of the trial drugs was determined.

RESULTS

Total four bacterial and two fungal species were selected in the present study to evaluate the anti microbial activity of the trial drugs. Different concentrations of the products were incubated and observed for the zone of inhibition. Antiseptic liquid did not provided any zone of inhibition upto the concentration of 900 mcg/ml. It implies that, the liquid doesn't have antibacterial and antifungal properties in the tried concentration. (**Table 4**)

The zone of inhibition with gel at the concentrations 400 mcg/ml was observed in two bacterial species (*B. pumillus* and *S. aureus*) found to be greater in comparison to reference standard antibacterial drug i.e. Ampicillin. The other two bacterial (*E. coli* and *Ps. aeruginosa*) and fungal species (*C. albicans* and *S. cerevisiae*) didn't responded to the maximum concentration (1500 mcg/ml) of the gel in current study. This indicates that the gel hand wash of *Panchavalkala* has anti bacterial activity particularly against *B. pumillus* and *S. aureus* at minimum concentration of 400 mcg/ml. (**Table 5**) the resistant organism may respond to the trial drugs in further concentrations.

Table 4: Result of antimicrobial study of antiseptic liquid:

Organism	Concentrations of drug					
	900(mcg/ml)	700(mcg/ml)	500(mcg/ml)	300(mcg/ml)	100(mcg/ml)	STD(mcg/ml)
Bacteria	Zone of inhibition (mm)					
E. coli	-	-	-	-	-	14.2
Ps. Aeruginosa	-	-	-	-	-	13.9
B. pumillus	-	-	-	-	-	16.4
S. aureus	-	-	-	-	-	15.1
Fungus						
C. albicans	-	-	-	-	-	13.3
S. cerevisiae	-	-	-	-	-	13.9

Table 5: Result of antimicrobial study of antiseptic gel:

Organism	Concentrations of drug									
	1500 (mcg/ml)	1000 (mcg/ml)	800 (mcg/ml)	600 (mcg/ml)	400 (mcg/ml)	200 (mcg/ml)	100 (mcg/ml)	50 (mcg/ml)	D (mcg/ml)	STD (mcg/ml)
Bacteria	**Zone of inhibition (mm)**									
E. coli	-	-	-	-	-	-	-	-	-	12.6
Ps. Aeruginosa	-	-	-	-	-			-	-	12.7
B. pumillus	19.2	18.9	18.5	18.1	17.7	16.7	14.2	-	-	17.2
S. aureus	17.0	16.7	16.5	16.3	15.7	15.5	13.4	-	-	15.0
Fungus										
C. albicans	-	-	-	-	-				-	13.5
S. cerevisiae	-	-	-	-	-				-	14.1

DISCUSSION

In order to provide improved elegancy, acceptability with increased shelf life, innovations are necessary in every field and *Ayurvedic* products are not spared from this. The present study is an attempt to convert *Ayurvedic* formulation (*Panchavalkala Kwatha*) into a ready to use antiseptic gel, which can be used as hand wash.

The microbial organism was found to be resistant to liquid samples. Probable cause for this inactivity may be the lower concentration of *Panchavalkala* in the end product. The gel showed significant results at concentrations starting from 400 mcg/ml against two bacterial species. The significance was found to be more in comparision to the standard reference. The composition (*Panchavalkala*) has been attributed with properties like free radical scavenging, anthelmintic, antimicrobial, anti inflammatory and analgesic etc. More concentrations may be needed to get a broad spectrum activity of the trial drug.

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