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Effect of gel based polyherbal handwash of azadirachta indica, citrus limonis, and aloe vera, on staphylococcus aureus,candida albicans and bacillus subtilis.

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Abstract
Infectious diseases that can be spread from person to person in homes and communities continue to be a major cause for worry. The changing nature and range of pathogens we are exposed to and community demographic changes that affect our resistance to infections are the main reasons for this. Both of these factors contribute significantly to the problem. It is essential to practice good hand hygiene in order to cut down on the spread of infectious diseases in both the household and the community. Because antibacterial soaps contain triclosan, which has a significant number of drawbacks, and alcohol-based sanitizers, which cause discomfort due to skin drying, we should consider replacing them with herbal antimicrobial components instead. The gel base is both the material and the process. Azadirachta indica, Citrus limonis, and Aloe vera are the herbs that are utilized in the preparation of polyherbal hand wash. This helps to prevent any unwanted side effects. The gel base polyherbal hand wash that was developed was tested for its antibacterial activity using the cup plate method, and the results were positive. Staphylococcus aureus, Candida albicans, and bacillus subtilis are the three types of microorganisms that are employed in antimicrobial testing. As a result of the experiment, we can draw the conclusion that gel-based polyherbal hand washes provide a higher level of inhibition than commercially available synthetic hand washes

Key words: Gel based Hand wash, Herbal, Antimicrobial activity, Non-toxic and Safe

Introduction

Because the hands are the primary method through which microbes and infections are spread, maintaining good hand hygiene is the single most critical action that can be done to both stop the spread of infectious diseases and protect oneself from contracting them. Proper sanitation of the hands is the single most

important, straightforward, and cost-effective method for reducing the spread of nosocomial infections (Ravi K, Pratibha MD et al 2005). Since most microorganisms and infections are communicated by direct contact, the most important thing someone can do to prevent the spread of disease and to protect themselves from being sick is to practice good hand hygiene. (Borgatta L, Fisher.M et al 1989). Washing one's hands thoroughly with soap and water is the single most effective, uncomplicated, and economical way to prevent the spread of nosocomial illnesses. Washing one's hands frequently is an essential part in preventing the transmission of disease. Washing one's hands not only removes the dirt that can be seen on them but also reduces the amount of potentially hazardous bacteria. People, animals, and even objects like kitchenware are all potential vectors for the spread of pathogenic bacteria and viruses like E. coli and Salmonella, which can then contaminate food (Kolhapure S.A 2004). The principal method of transmission of multidrug-resistant bacteria and illnesses to patients is through the hands of health care workers (HCWs). Because of this, it raises the issue of employing disinfectants when washing one's hands (PittetD et al 1999). Sanitizers with an alcohol base can now be found in many of the chemical antiseptics that were previously only available. These soaps or solutions serve to minimize the transmission of infectious diseases that are related with healthcare in a more effective manner (Arya M, Arya P et al 2005). However, they are not without certain drawbacks or negative effects. Regular use of these products may cause irritation to the skin as well as resistance in disease-causing microorganisms (Winnefeld M, Richard MA, et al 2000). There are many different organisms that can cause skin infections, but some of the more common ones are Staphylococcus aurous, Pseudomonas spp., Bacillus subtilis, and Candida albicans (Rotter M 1999).

Neem trees, also known as Azadirachta indica, are native to Indonesia and may be found in a number of different regions there, including Bali, Lombok, West Java, Central Java, and Nusa Tenggara Barat (Ragasa C.Y., Nacpil Z.D et al 1997).

Originating in the Meliaceae family, this plant is also known as Indian lilac or Margosa. In India, numerous components of the neem tree have been included into the practice of traditional Ayurvedic medicine. Quercetin is the primary flavonoid and nimbosterol is the primary ß-sitosterol that is found in neem leaves, in addition to a number of liminoids (nimbin and its derivatives). It is well known that quercetin, which is a polyphenolic flavonoid, possesses antibacterial and antifungal effects. In traditional Indian medicine, neem oil, bark, and leaf extracts have all been used therapeutically to treat a variety of conditions, including leprosy, intestinal helminthiasis, respiratory diseases, and constipation. They have also been used to promote overall health. Neem oil has been shown to be effective in treating a variety of skin ailments. It has been reported to possess Antibacterial, antifungal, antiviral, anticancer, antioxidant, anti-ulcer, antifertility, and hypoglycemic properties are all present. Blood diseases, biliary disorders, itching, skin sores, and burns are all alleviated by using the bark, leaf, root, flower, or

fruit. (Chatterjee A, Pakrashi S 1994, PrarthanaThakurta, Poulami Bhowmik et al 2007, Parida MM, UpadhyayC et al 2002, Raveendra M Pai, Leelavathi Acharya D 2004, Saradhajyothi Koona, SubbaraoBudida 2011)

Aloe Vera: The plant Aloe barbadensis, which is a member of the Lilliaceae family, is where the gel that is used to make aloe vera products comes from. It has been utilized historically for cosmetic purposes as well as for its wound healing abilities. It contains chemical elements such as antheracene glycosides, acemanna and anthraquinones, all of which demonstrate anti-microbial activity against harmful bacteria. Other activities, such as antifungal, anti-diabetic, anti-inflammatory, anticancer, immunomodulatory, and gastro protecting characteristics, have been reported, and these activities encourage its application in the pharmaceutical, cosmetic, and food industries (Hamman Josias et al 2008 Ramasubramanian V, et al 2011). It has been suggested that the aloe vera gel contains mannose-6-phosphate, which has been shown to have a wound healing effect (R. H. Davis, J. J. Di Donato)

Juice obtained from the fruits of Citrus limon, which are members of the Rutaceae family, is known as lemon juice. The primary components of the fruit juice are fruit acid, citric acid (18%), and sugar. The lemon fruit's inner layer is rich in a variety of coumarin derivatives and bitter flavones glycoside(Gov. of India, Ministry of Health and Family Welfare), among other compounds. Because of the disinfectant characteristics it possesses, the primary application for it is cleaning. In some culinary dishes, lemon juice serves the additional function of acting as a preservative for a shorter period of time. Because of the lemon's natural anti-microbial capabilities, Indian medicinal systems make use of lemon juice as an ingredient. Additionally, it is utilized in the culinary industry as a flavoring agent (Dhanavade Maruti J, et al 2011) . The metabolic extracts of Azadirachta indica were used in the preparation of the hand wash, together with lemon juice and aloe Vera juice. Plant extract shown to have antimicrobial activity

Both the cosmetic and pharmaceutical industries are becoming increasingly interested in finding natural alternatives to synthetic antimicrobials found in topical solutions. Not only is there a growing interest among consumers for natural agents, but there is also an increase in microbe resistance to traditional antimicrobials (Augustin, M.; Hoch, Y 2004) Phenolic chemicals are found in plants, and plants also produce them themselves as part of their defense mechanisms. They can have an effect by interacting with the cell membrane or cell wall of the microorganism, which can result in changes in the permeability of the membrane and ultimately lead to the death of the cell (Cowan, M.M 1999, Taguri, T.; Tanaka, T 2006, Tian, F.; Li, et al 2009). Phenolic can also enter bacterial cells and cause the contents of those cells to coagulate, thereby killing the bacteria. Because they act as natural antimicrobials, phenolic compounds have the potential to extend the shelf life of a variety of different items by preventing the growth of dangerous bacteria(Rains, J.L 2011) [23].

Material And Methods

The present research work have been execute at Department of Pharmacology & KIMSDTU, Krishna Institute of Pharmacy, Malkapur, Karad, MS, India. The collection and processing parts of plant material was acquire & authenticated by a taxonomist. All the chemicals and reagent used for this consider were purchased research–grade quality and procure in our laboratory according its preservative conditions.

Collection of plant material and preparation of extracts:

The plant materials were gathered at a market in the immediate area. The Department of Botany of the Y. C. Institute of Science in Satara was responsible for both the taxonomic identification and the authentication. The methanolic extracts of Azadirachta indica, the juice of one lemon, and the juice of one aloe vera leaf were used to make the hand wash. Through the process of extraction, 20 grams of powdered neem plant leaves were processed using 100 milliliters of a methanol solution that had nine parts methanol to one part distilled water. This combination was put through a five or six hour long hot continuous extraction process using a Soxhlet device. After the extraction step was finished in its entirety, the solvent was evaporated, leaving behind a concentrated dry residue. Overnight, herbal gel was made by combining 1% weight-for-weight concentration of carbopol-934 with deionized water. The gelling agent used was carbopol-934. After that, a mechanical stirrer was used to mix the expanded polymer in order to guarantee that it was evenly dispersed throughout the solution. The pH adjusted to 7.0 with triethanolamine in extremely trace amounts while the mixture was continuously stirred. After dissolving the methanolic extracts of Azadirachta indica in 6-8 milliliters of methanol in a mortar and pestle, add 4 milliliters of lemon juice to 20 milliliters of distilled water, followed by the appropriate amount of aloe vera juice and sodium lauryl sulfate according to the above table. The aforementioned gel mixture should then be added to the mortar and pestle together with honey, peppermint oil, any scents or flavoring agents, and an enough amount of preservative. The formula for the gel used for washing hands was created in accordance with Tables 1 and 2. After that, the formulation was tested for its organoleptic properties.

Table 1: Formula for Gel Base hand wash containing herbs

Components	**Amount**	**Use**
Triethanolamine	Qty. Sufficient	Neutralize the acid
Cabopol-940	300 mg	Formation of Gel
Purified Water	0.020 l	Act as a Vehicle

Table 2: Formulation of Hand Wash gel based formula

Ingredients	Quantity Taken	Role
Gel base	20ml	To increase viscosity
Extract	2gm	Kills bacteria
Juice of Lemon	4ml	Cleaning activity
Juice of Aloe Vera	5ml	Curing activity
Honey	5 ml	Humectants, Moisturizer
SLS	0.7gm	Surface active agent
Oil of peppermint,	0.2ml	Agents for flavoring
Oil of Orange peel	0.5ml	Agents for cooling

Evaluation

Physically Parameters: Color, Odour appearance and homogeneity was decided by Visual inspection

Chemical Parameters

A) pH : The use of a pH meter allows for precise measurement of the value of pH.

B) Viscosity

The digital Brookfield viscometer was used to measure the thickness of Poly Herbal Gel Based hand wash. A sample of Poly Herbal Gel Based Hand Wash (0.5 g) was diluted with 25 ml of distilled water and tested for

C) Foam Height. Then, 50 ml of water was added to bring the total volume to 500 ml in a measuring cylinder with a stopper. After 25 strokes, the aqueous volume reached 50 ml, and the foam height was found to be 50 millimeters higher.

D) Retention of Foam: After placing 50 ml of the Poly Herbal Gel Based Hand Wash into a 200 ml graduated cylinder, it was shaken vigorously for 10 seconds. Foam volume was measured at 1-minute intervals for 4 minutes. At least 5 should pass with no change in foam retention.

Anti Microbial Studies

The cup plate method was used to test the antimicrobial efficiency of the prepared poly herbal hand wash and extracts on a variety of microorganisms in accordance with the standard operating procedure.

For the purpose of evaluating the antimicrobial activity against three distinct microorganisms, Candida albicans, Staphylococcus aurous, and Bacillus subtilis species, three sterile petri plates were used. After the sterile nutrient agar solution had been spread throughout the plates, the solidification process was allowed to take place. After the solidification process was complete, the bacteria from the subculture were sterilized and then introduced into the nutrient agar

media. Additionally, two cavities were created within the nutritional agar. The first cavity is used to conduct an experiment with a gel-based polyherbal hand wash, while the second cavity is used to conduct an experiment with a synthetic hand wash solution that is commercially available. It was ensured that the sample would be positioned at the level of the cavity after it had been filled with 0.1 milliliters of the hand wash solution. In order to evaluate the level of activity, the plates are kept in an incubator at 370 degrees Celsius. After a period of 24 hours, the plates were examined to see whether or not a zone of inhibition had formed. An estimate of the anti-microbial activity of the formulation can be derived from the zone of inhibition.

Results And Discussion

Formulation was evaluated as per physical and chemical parameters results shows brown colour, homogenicity was good, it was observed chemical parameters as per table no.3.

Table 3: Evaluation of Polyherbal hand wash

Sr.No.	Test	Observation
1)	Physical parameter	
	Colour	Brown
	Odour	Characteristics
	Appearance	Translucent
	Homogenicity	Good
2)	Chemical Parameter	
	P^H	6.7
	Viscosity	45 cPascal's
	Foam Height	290 ml
	Foam Retention	22.5

Antimicrobial activity screening tests

By using the cup plate method, an anti-microbial efficacy test was performed on Candida albicans, Staphylococcus aureus, and Bacillus subtilis to determine the effectiveness of the prepared Herbal Hand Wash. The findings of the cup plate technique revealed that the handwash that was made from the methanol extract of the combined plant materials exhibited more activity than the activity of the commercially available hand wash that was given in table 4.

Table 4: Zone of Inhibition

Sr. no	Micro organism	Zone of inhibition of Std. hand wash(mm)	Zone of inhibition of Test hand wash(mm)
1.	S.A	23	39
2.	B.S	25	45

3.	C.A	30	42

S.A - Staphylococcus aureus, B.S. - Bacillus subtilis, C.A. – Candidaalbicans

Plates showing zone of inhibition

S:-Standard sample.

T:-Test sample.

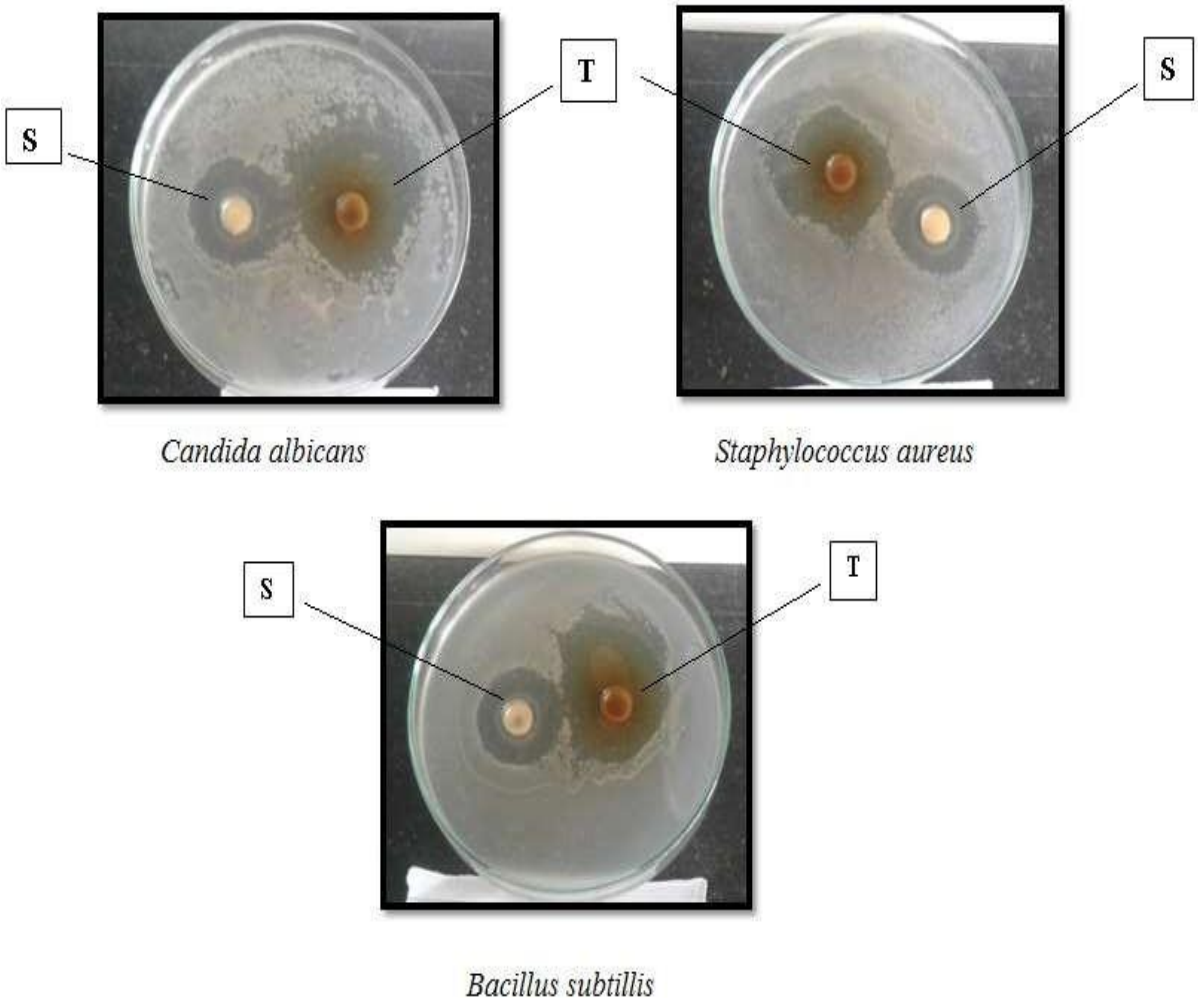

Figure 1: Photographs of Antimicrobial Activity

Conclusion

The extracts of Azadirachta indica, Citrus limonis, and Aloe vera, as well as their mixtures, suppress skin pathogens better than antiseptic hand wash. Herbs in hand wash may make sense. The cup plate technique was used to evaluate the herbal hand wash. The homemade herbal hand wash outperformed the commercial synthetic hand wash. Thus, these chemicals may be isolated and integrated into bases to provide a better anti-microbial hand wash with fewer or no adverse effects owing to a lower surfactant and alcohol content than current formulations. These formulations have a greater concentration of these substances. Thus, a novel approach may be found to bring back antibiotic-resistant harmful organisms and give safe and healthy life via germ-free hands. Though not 100%, a significant

percentage may be reduced.

It was discovered that a polyherbal gel base hand wash formulation had positive effects and outstanding efficacy against all of the bacteria that were tested. As a result, the fact that it was effective as an antiseptic preparation was quite encouraging.

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Conflict of interest:

The authors declare no conflict of interest.

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