



# Formulation and Evaluation of Polyherbal Hand Sanitizer

Rina Maskare\*, Nitin Indurwade, Abhishek Purohit, Veena Atrahe  
Manoharbai Patel Institute of Pharmacy (B.Pharm), Kudwa, Gondia,  
Maharashtra.441614

Received: 02 Jul 2019 / Accepted: 9 Aug 2019 / Published online: 1 Oct 2019

Corresponding Author Email: [rinamaskare@yahoo.co.in](mailto:rinamaskare@yahoo.co.in)

## Abstract

**Objective:** The main objective of the work was to perform extraction and formulation of polyherbal hand sanitizer is for “hand hygiene”. It is a vital principal in the prevention, control, and reduction of any acquired infection. **Method:** The fresh leaves of *Azadirachta indica* and *Eucalyptus globulus* were collected and then the plants were authenticated, collected leaves were washed, shade dried and used for the further investigation. The dried plant sample was grinded using mechanical grinder to fine powder. Various phytochemicals were extracted by hydroalcoholic solvent extraction using Soxhlate apparatus and the extracted phytochemicals were qualitatively analyzed prior to study of the antibacterial activity. This herbal combination of extracts was used for formulation of hand sanitizer. **Results:** The efficacy of herbal hand sanitizer was evaluated using microorganism suspensions (Bacteria- *E. coli*, *Staphylococcus aureus*); which revealed that the herbal hand sanitizer is efficient in reducing higher number of microorganisms from the hands as compared to commercial synthetic hand sanitizer. **Conclusion:** The higher antimicrobial activity and efficacy of these herbal extracts can be used in the preparation of herbal hand sanitizers on commercial scale. The ingredients on combination behave as an effective hand sanitizer.

## Keywords

Antimicrobial Activity, *Azadirachta indica*, *Eucalyptus Globulus*, Hand Hygiene, Herbal Sanitizer, Hydroalcoholic Extraction, Minimum Inhibitory Concentration.

\*\*\*\*\*

## INTRODUCTION

One of the most important scientific reason and religious belief is to wash hands before eating the meals. This is to avoid the unwanted and undesirable microorganism to enter the body and cause infectious diseases.

Most of the hospital acquired infections are caused due to the unhygienic condition of the hands of both the patient and the hospital staff. It is therefore the strict instruction of the medical council and W.H.O to clean the hands by the approved hand sanitizers available in the hospital. Because of the poor hand hygiene, the people suffer from majority of nosocomial infections including gastrointestinal and respiratory infections. Thus, it is important to

decontaminate the hands using safe water or wearing gloves. However, it is not always possible to make available the safe water and the gloves which are present only in the hospitals. Thus, the best and easily available source to disinfect the hands is the use of hand sanitizers which can be easily installed in hospitals, laboratories, restaurants and in toiletries [1,2].

The common opportunistic pathogens which cause hospital acquired infections are *Staphylococcus aureus*, *Escherichia. Coli* and *Pseudomonas aeruginosa*.

The most common use to disinfect the hands is to wash it with clean water. However, the water used may not be safe all the times and therefore the use

of soap and detergents have been introduced in addition to the water. Still in recent times the hand sanitizers have been introduced in the market which claims to have great bactericidal activity and safe for use.

The hand sanitizers available in the market are both alcohol based and non-alcohol. The alcohol-based hand sanitizer claims to kill 99.99% microorganisms including the most resistant form.

The hand sanitizers are available in the form of liquid, foam or easy flowing gel formulations, which can be applied on palm of the hand, rub the product over all surfaces of hands and fingers until hands are dry. The product is widely used by the doctors, surgeons before and after the surgery, pathologists, and researchers and is also used at restaurants, toiletries etc. The medical and applied medical science colleges in their laboratories also have hand sanitizer which the students use after every practical class.

The aim of the present study was to prepare hand sanitizer formulation using the extracts of *Azadirachta indica* (Neem) and *Eucalyptus globulus* (Nilgiri) and to investigate the antimicrobial activity of the extracts against the common organisms which cause nasocomial infections. Furthermore, to evaluate the stability and phytochemical parameters of the prepared formulations so that they can be further standardized and used commercially.

## MATERIALS AND METHODS

Extraction of neem (*Azadirachta indica*), and eucalyptus (*Eucalyptus globulus*) were extracted in MIBP, Gondia. Ethanol, methanol, sorbitol, polysorbate 20 was obtained from (S D FINE-CHEM

LIMITED, Mumbai, India). All other chemicals used were of analytical grade.

Bacterial Strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis* were procured from D.B.Science college, Gondia.

### Collection, Drying of Plant Material:

Mature plants of *Azadirachta indica* and *Eucalyptus globulus* were used for this preparation and were collected from Gondia city in 2019. The leaves were collected, dried in shade, coarsely powdered and well-stoppered container. The dried material of *Azadirachta indica* and *Eucalyptus globulus* was then used further work.

### Method for extraction of neem extract:

Preparation of plant extract 10g of powdered leaves were extracted successively with 100 ml of methanol at 40-50°C in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use [3].

### Method for extraction of Eucalyptus extract:

Extraction was carried out by soxhlet apparatus and it's working on steam distillation process. The powdered leaves of 50 gm were added on 400 ml. of ethanol, the working temperature was maintained at 40°C and distillate for 1 hour. Once the distillation started the sample start boiling within 5 min and vapour are formed. The vapour is cooled down with the help of condensed. The condensed material was collected on the other side of setup. After completion, the extraction process, the solvent and extractor were placed rotary evaporator to evaporate the solvent [3].

### Quantitative yield of extract:

$$\text{Percentage yield} = \frac{\text{Weight of the sample extract obtained (g)} \times 100}{\text{Weight of the powdered sampled used (g)}}$$

### Preliminary antimicrobial screening of the extracts

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, glycosides, terpenoids and steroids, flavonoids, reducing sugars, triterpenes, phenolic compounds and tannins. All the extracts of leaf were subjected to preliminary antimicrobial screening by dilution method against the organisms *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa*. The extracts which exhibited maximum activity were selected for the formulation [4].

### Method of Preparation of Hand Sanitizer:

Extracts and other ingredients were accurately weighed. The quantity of denatured alcohol and

water were separated in four equal quantities. In each part of denatured alcohol, both extracts, emollient and preservative were uniformly mixed using mechanical stirrer. To this four-alcoholic mixture, four equally separated quantities of deionized water were added and mix until the mixture was uniform. With continuous stirring, the solution of both the extracts was mixed together and the solution of emollient was also added to this, which is followed by the addition of preservative solution. These solutions were uniformly mixed, and emulsifier was added. After ensuring the solutions are mixed properly to one another perfume was added [5,6,7]. The composition is given in Table no.1.

**Table 1. Composition of hand sanitizer**

Sr. No.	Ingredients	Quantity given (%)	Quantity taken (gm/ml)	Uses
1	Eucalyptus extract	1.5	0.7	Antimicrobial
2	Neem extract	1.5	0.7	Antimicrobial
3	Sorbitol	2.30	2.12	Emollient
4	Polysorbate 20	0.50	0.48	Emulsifier
5	Methyl paraben	0.50	0.3	Preservative
6	Alcohol denatured	62.0	15.5	Antibacterial
7	Deionized water	30.0	10	Vehicle
8	Perfume	Q.S.	Q.S.	Fragrance

**Evaluation of physicochemical parameters of the hand sanitizer:**

The physicochemical characteristics of hand sanitizer such as clarity, colour, odour, were determined as per Indian Pharmacopoeial Procedures and pH was determined using digital pH meter. The foam height, foam retention, alcohol insoluble matter was determined [5].

**Stability of formulation:**

Liquid sanitizer was allowed to stand at 37°C for two months. The stability of liquid sanitizer was observed during this period. The sample which was stable liquid after standing was indicated as stable and the sample in which precipitation were caused; then liquid was said to be as unstable.

**Antimicrobial testing of the prepared formulations:**

The screening of antibacterial activity of the formulation against pathogens was performed using disc diffusion method. Nutrient agar media was prepared, sterilized and aseptically spread on four sets of Petri plates which were previously marked as formulation coding. Microorganisms used were *Staphylococcus aureus*, *Escherichia coli*, *B.subtilis*, *P.aeruginosa*. The plates were inoculated with microorganism suspension and incubated at 37°C for 24 h. Next day filter paper discs loaded with alcohol based herbal hand sanitizer and synthetic hand

sanitizer were placed in the respectively marked plates. It was taken care that the sterile discs completely absorb the formulation. After 24 h test results were observed to determine the efficacy of formulations in terms of zone of inhibition of microorganism. Higher the zone of inhibition, the more effective is the test formulation [1,2,6].

**RESULTS AND DISCUSSION**

In the present study ethanolic extract of *azadirachta indica* and methanolic extract of *Eucalyptus globulus* were taken for the formulation. These extracts were evaluated for its preliminary phytochemical screening. The results are given in table no.2 and 3. The % yield was found to be 14% and 16% for *Azadirachta indica* and *Eucalyptus globulus* respectively. The methanolic and ethanolic extract showed good antibacterial activity against *E. coli*, *S.aureus*, *B.subtilis* with 1.56 mg/ml, 3.12mg/ml, 3.12mg/ml and 1.56mg/ml, 3.12mg/ml, 3.12mg/ml and mild to moderate antibacterial activity against *P.aeruginosa* with 6.25mg/ml and 12.5mg/ml respectively. The results are given in table no.4. The prepared formulations were evaluated for physicochemical parameter and antimicrobial activity. The results are shown in Table 5 and 6 respectively.

**Table no. 2. Preliminary phytochemical screening of *Azadirachta Indica*:**

Sr.no	Test	Result
1	Phenolic compound	+
2	Terpenoid	-
3	Triterpenes	-
4	Tannins	-
5	Saponins	-
6	Steroids	+
7	Flavonoids	+

Absent: (-)      Present: (+)

**Table no. 3. Preliminary phytochemical screening of *Eucalyptus globulus*:**

Sr.no	Plant constituents	Test / reagent	Result
1	Tannins	Ferric Chloride	+
		Lead Acetate	+
		Pot. Dichromate	+
2	Sterols	Salkowaski	+
		Liebermann's	+
		Liebermann-Burchard	+
3	Terpenoids	Phenol	+
4	Alkaloids	Dragendorff's	-
		Hager's	-
		Mayer's	-
		Wagner's	-
5	Flavonoids	Shinoda Test	+
6	Saponins	Foam Test	+
7	Glycosides	Anthraquinone glycosides	-
		Cumarin test	-

Absent: (-)      Present: (+)

**Table no.4. Antibacterial activity of *Azadirachta indica* and *Eucalyptus globulus* (Minimum Inhibitory Concentration):**

Sr. No.	Plant extract	Microorganism	Concentration (mg/ml)										MIC (mg/ml)	
			50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.2	0.1		
1	<i>Azadirachta indica</i>	<i>S.aureus</i>	++	+	+	-	-	-	-	-	-	-	-	3.12
		<i>E. coli</i>	++	+	+	+	-	-	-	-	-	-	-	1.56
		<i>B. subtilis</i>	++	+	+	-	-	-	-	-	-	-	-	3.12
		<i>P. aeruginosa</i>	+	+	-	-	-	-	-	-	-	-	-	6.25
2	<i>Eucalyptus globulus</i>	<i>S.aureus</i>	++	+	+	-	-	-	-	-	-	-	-	3.12
		<i>E. coli</i>	+	+	+	-	-	-	-	-	-	-	-	1.56
		<i>B. subtilis</i>	++	+	-	-	-	-	-	-	-	-	-	3.12
		<i>P. aeruginosa</i>	+	+	-	-	-	-	-	-	-	-	-	12.5

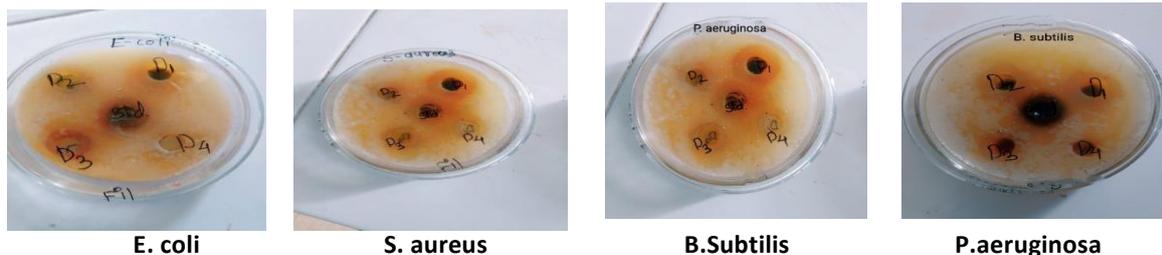
Turbidity: Present = (+)      Absent = (-)

**Table no.5. Physicochemical parameters of formulations**

Sr.No..	Parameters	HF1	HF2
1	Colour	Dark green	Dark green
2	Odour	Aromatic	Aromatic
3	Appearance	Liquid	Liquid
4	pH	6.46	6.00
5	Foam height	5.0	4.5
6	Foam retention	1.5	1.2
7	Alcohol insoluble matter	2.3	3.1
8	Stability	Stable	Stable

**Table no.6. Antimicrobial screening of the prepared formulation**

Organism	Zone of Inhibition (Diameter in mm)				
	Conc. Of Drug in $\mu\text{g/ml}$				
	800	600	400	200	Std.
E. coli	36	34	32	30	37
S. aureus	34	32	32	28	32
B. subtilis	32	30	28	28	30
P.aeruginosa	30	28	25	20	31

**Fig.1: Antimicrobial study of prepared formulation against various pathogens**


The main objective of the present study was to prepare herbal hand sanitizer and to investigate whether the formulation show a antimicrobial activity against the common organisms which cause nasocomial infections. The prepared formulation of herbal hand sanitizer showed significant results at concentrations starting from 400  $\mu\text{g/ml}$  against four bacterial species (E. coli, B.subtilis, P.aeruginosa and S.aureus). The significance was found to be more in comparison to the standard reference. The composition (Azadirachta indica and Eucalyptus globulus) has been attributed with properties like free radical scavenging, antimicrobial, anti-inflammatory and analgesic etc. More concentrations may be needed to get a broad-spectrum activity of the test drug. The alcohols have excellent, rapid (within seconds) germicidal activity against vegetative bacteria, fungi, and many viruses and antimicrobial activity is based on protein denaturation of microorganisms. Alcohol sanitizers are highly effective against mycobacteria (the bacteria most resistant to the disinfection process) and multidrug-resistant pathogens. Alcohol rubs are approximately 100 times more effective against viruses than any form of hand washing. Sanitizers offer numerous advantages over non-alcoholic hand disinfectants as, rubbing sanitizers onto both hands & until it completely evaporates usually require only 15 to 30 seconds. Whereas vigorous friction, rinsing with water, and drying with a towel are not needed like hand disinfectants or soaps.

#### CONCLUSION:

Hands are the most common mode of transmission of pathogens to patients and proper hand hygiene

can prevent health care-associated infections and the spread of antimicrobial resistance. Scientific evidence and ease of use support of alcohol-based hand sanitizers during patient care. It may be concluded that Herbal Hand Sanitizer has a significant anti-microbial effect on the specified microorganisms except Ps. aeruginosa and S. cerevisiae. Thus, there is immense potential in establishing the use of antimicrobial herbal products as a measure to control the multidrug resistant microbes as well as check their spread through hands from one geographical region to another.

#### REFERENCES:

- [1] Otokunefor, K; Princewill, I, Evaluation of antibacterial activity of hand sanitizers – an *in vitro* study, J. Appl. Sci. Environ. Manage. Dec, 2017, Vol. 21 (7) 1276-1280.
- [2] Dr. Yousuf Adam Ali, to study the effect of hand sanitizers used in kingdom of saudi arabia against the common bacterial pathogens, International Research Journal of Natural and Applied Sciences Volume-2, Issue-2 (February 2015),17-28.
- [3] Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy, 51<sup>st</sup> Ed., Nirali Prakashan, Pune, 2015, P. 1.1,14.41-14.43.
- [4] Seerangaraj V., Suruli K, Konda Mani Saravanan, Preliminary Phytochemicals Screening, Antibacterial potential and GC-MS Analysis of medicinal plants extracts, Article in journal of pharmaceutical sciences. April 2016.
- [5] Zeeshan A., Salma K., Formulation and Evaluation of Poly Herbal Hand Sanitizer, Article in International Research Journal of Pharmacy, September 2016.
- [6] P.P Sharma, Cosmetics –Formulation, Manufacturing and Quality Control IV edition, Vandana Publication, Delhi ,2008 P.143-148.

- [7] Sanju N., Arun N., Roop K, Cosmetic Technology, I edition, Brila Publication, Delhi, 2009-10 P.115-117.
- [8] Rangari VD. Pharmacognosy and Phytochemistry, Vol I, 2<sup>nd</sup> Ed., Career Publications, Nashik, 2008, P. 204,346.
- [9] Ali M. Pharmacognosy and phytochemistry, Vol I, CBS Publishers and Distributors, New Delhi, 2009, P.462-463, 676-683.
- [10] Khadabadi SS, Deore SL, and Baviskar BA. Experimental Pharmacognocny 1<sup>st</sup> Ed., Nirali Prakashan, Pune P. 3.1-3.8.
- [11] Tortora, F.C, Microbiology an Introduction IX edition Pearson Publication. P.602.
- [12] Dr. Suresh Jain, Professor Vipin Saini, Dr. Naitikkumar, A Practical Book on Calibration Analytical Instruments III edition 2015, Nirali Prakashan. P. 10.
- [13] Chandrakant Kokare, Pharmaceutical Microbiology, Experiments and Techniques, Career Publication II Edition. P.84-86, 138-139.