

Formulation and Evaluation of Herbal Hand Sanitizer

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ABSTRACT:

Infection causing microbes spread through the air, water, contaminated food, cloth, equipment or from infected person to healthy one. The evidence collected on spreading mode of COVID-19 is mostly through skin contact and respiratory droplets. However, just in contact with the microbe is not enough to cause the disease. Reaching the germ to the internal body is the critical step involved in the manifestation of illness. Contact transmission for the sign of disease in an individual can occur from skin to the inner body especially when the infected part (mostly hands) reaches the mucous membrane of open cavities such as mouth, nose, eyes or open wound. Therefore the use of good quality sanitizers to prevent infections such as COVID-19 is one of the critical measures. Ayurveda is a rich source of vast information on therapeutic potentials of numerous herbs. Many simple to complex formulations can be prepared by using the therapeutic attributes of these herbs. Therefore in the present work, an attempt has been made to initiate the manufacturing of Ayurveda hand sanitizers based on various Ayurvedic medicated drugs mentioned in Samhitas, as developing countries such as India are facing a shortage of hand sanitizers which is a must prevent the spread of COVID-19. The shortage has resulted in an internal price hike, and thus there is a need for a homemade affordable way of sanitizing hands. Therefore by using a combination of antimicrobial, disinfectant, anti-allergic, skin disease curing herbs along can be recommended for the preparation of Ayurveda hand sensitizers. By using similar herbs, nasal spray and aerosols for cleaning and increasing the strength of the respiratory tract can also be prepared.

1. Introduction :

Hygiene is defined as the maintenance of the practice of cleanliness which is of most importance in the maintenance of well-being. Keeping body hygiene and using the cleanser are necessary for a healthy life. These concepts underscore the need to maintain hygiene in disease prevention(1)

In the current scenario of mechanized life style; a consumer will always prefer ready-made formulation of alcohol hand rub rather than hand washing (application of a non-antimicrobial or antimicrobial soap; and mechanical friction is generated by rubbing the hands together for 1 minute, followed by rinsing with water, and then drying thoroughly with a disposable towel).(3)

Traditional healers have long used plants to prevent or cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Considering this ultimatum; an attempt has been made to screen classical literature for the herbs with antimicrobial properties and found that *Mentha piperita* (Peppermint leaves), *Eugenia caryophyllus* (Clove flower buds) and *Azadirachta indica* (Neem

leaves) has those antimicrobial activities. To formulate and evaluate herbal sanitizer comprise of combination of alcoholic extracts *Azadirachta indica* (Neem) and *Mentha piperita* (Peppermint) along with oil of *Eugenia caryophyllus* (Clove) using suitable excipients; which can be used as a ready-made herbal hand sanitizer.(7)

The emergence of novel pathogens, bacterial or viral, has always posed serious challenges to public health around the globe. One of these dangerous pathogens is “severe acute respiratory syndrome coronavirus 2” or SARS-CoV-2, more commonly known for causing coronavirus disease 2019 or COVID-19, which has been declared a global pandemic by the World Health Organization in early 2020. Since its discovery in December 2019 in Wuhan, there have been over three million confirmed cases worldwide by April 2020.¹ With cases increasing exponentially around the world, it has caused significant burden on all aspects of society despite aggressive isolation methods to prevent the spread of the virus. Currently, therapeutic strategies to deal with COVID-19 are only supportive, making prevention aimed at reducing transmission the best method at this time.(2)

One of the many ways implemented to prevent the spread of this virus, as with previous contagious pathogens, is frequent and effective handwashing. In both healthcare and community settings, alcohol-based hand sanitizers have become a popular alternative to the traditional handwashing with soap and water. Alcohol-based hand sanitizers have been utilized as an effective alternative to handwashing to prevent the spread of bacterial and viral infections, making it one of the essential protocols in decreasing healthcare burden. A range of hand sanitizers are available with various combinations of ingredients and modes of delivery. Given the popularity of hand sanitizers during this pandemic, it is important to understand which types of hand sanitizers work best against this novel virus. In this review, we will discuss the role of various types of alcohol-based hand sanitizers in effective elimination of bacterial and viral pathogens(4,5)





Fig:Examples of herbal Hand Sanitizer

2. LITERATURE SURVEY :

Akrayi (2014) : *Ocimum sanctum* (family Lamiaceae) an aromatic plant of the Indian subcontinent, and used in Ayurvedic system as an antipyretic, antibacterial, antihelminthic and fungicidal. Different parts of the plant to be useful in many diseases. Citrus lemon (family Rutaceae) is also a useful medicinal plant cultivated mostly for its alkaloids and have antibacterial potential in its parts viz, leaves, stem, root and flower. Significant bactericidal action of this plant has been reported along with anti-fungal anti-diabetic, anticancer and antiviral activities. Therefore Kwatha (medicated decoction) of these medicinal herbs can be formulated into an antiseptic liquid which further can be used as a readymade hand sanitizer. Likewise, Nasal spray can also be prepared by using these drugs, which are effectively useful in respiratory tract diseases.

Hammer, Carson & Riley (1999) ; All parts of *Azadirachta indica* possesses therapeutic potentials and useful in treating skin diseases, scalp diseases, hair loss, impaired liver function; and also act as a blood purifier, anti-inflammatory, anti-diabetic, antiviral, anti- carcinogenic, immune-modulatory property etc

Dimic et al (2012) : The concept of hand disinfection by using antiseptic agent probably emerged in the early 19th century. Before the discovery of modern medicine, plants were the primary remedy for treating various diseases. With the advent of different antibiotics, microbes also gradually develop resistance to these substances. These bring researchers to interest in the plants having antimicrobial properties. Readily made sanitizers are feasible for use as they can be carried easily and does not have any serious adverse reaction on the skin.

Ravi, Pratibha, & Kolhapure (2005): Proper hygiene, particular hygiene of hand⁵ can be considered as one most important, most straightforward as well as a highly affordable measure for preventing the spread of resistant microbes associated infections, especially nosocomial infections. Therefore every person engaged inpatient care should be careful and well aware of correct hand hygiene on time to time . Contaminated hands can also infect food and drink, which if consumed by a healthy person, then he/she will be affected by various systemic diseases). Therefore hand hygiene is a must for personal as well as to restrict community spread of infection.

3. AIM AND OBJECTIVE :

Aim : To study herbal hand sanitizer its manufacturing and evaluation.

Objective :

1. Hand sanitizers are convenient, portable, easy to use and not time consuming.

2. Several studies have concluded that the risk of spreading gastrointestinal (stomach) and respiratory infection is decreased among families who use hand sanitizers.
3. Commercially prepared hand sanitizers contain ingredients that help prevent .Using these products can result in less skin dryness and irritation than hand-washing.
4. Studies show that adding hand sanitizers to classrooms can reduce student absenteeism due to illness by 20 per cent. What's more, many kids think instant hand sanitizers are fun to use.

4. Herb's Use in Sanitizer :

Some herbs that are used to prepare herbal hand sanitizers are:

- A. *Ocimum sanctum* (tulsi leaves),
- B. *Eugenia caryophyllus* (clove),
- C. *Cymbopogon flexuosus* (lemon grass),
- D. *Aloe baarbadensis* (aloe),
- E. *Mentha arvensis* (mint),
- F. *Azadirachata indica* (neem),
- G. *Eucalyptus globulus* (eucalyptus).



Fig. Tulsi Leaves



Fig.Clove



Fig.Lemon Grass



Fig.Aloe



Fig.Mint



Fig.Neem



Fig.Eucalptus

5. FORMULATION OF POLYHERBAL HAND SANITIZER :

S.No	Ingredients	Quantity (%)
1	Ginger 	10
2	Lemon 	10
3	<i>Andrographis paniculata</i> 	10
4	Carbapol-940	1
5	Triethanoplamine	1
6	Glycerine	5
7	Polysorbate-20	1
8	Perfume	1
9	Preservative	1
10	Alcohol	40
11	Water	20

6. METHOD OF PREPARATION :

Ethanollic extract of *Andrographis Paniculata*, ginger, lemon was prepared by Maceration process. Other ingredients except triethanolamine were added in water and stirred well using a mechanical stirrer. To this the extracts were added and stirred. Then triethanolamine and perfume was added and the volume was made up using alcohol.

7. EVALUATION PARAMETERS :

Colour: It was determined visually.

Odour: It was determined manually

pH: It was determined by using pH meter.

8. Antimicrobial studies of herbal hand wash gel :

The screening of anti-microbial efficacy of the formulated poly herbal hand wash gel was aseptically performed on Escherichia coli, Staphylococcus aureus, and Salmonella by using Dip well Agar Diffusion Technique described by Bauer et al., 19669 and demonstrated by Cakir et al., 200410 was employed for antibacterial bioassay. A well was prepared in the plates (containing 15ml of Muller–Hinton agar medium) with the help of a cork-borer (0.85cm). 100 l of the test compound (herbal hand wash gel) was introduced into the well. The standard antibiotic discs like erythromycin, penicillin, streptomycin and ampicillin were used as a standard. The plates were incubated overnight at 37°C. Efficiency of hand wash gel was determined by measuring the diameter of zone of inhibition at 200 g ml-1, 400 gml-1 and 800 gml-1 concentration.(10)

9. Antibacterial efficiency of herbal hand sanitizer gel :

The antibacterial efficiency was performed by spread plate technique. Samples were collected from the five different volunteers showing no clinical signs of dermal abrasion, trauma and infection. Approximately 500 l of herbal hand wash gel was applied to both hands. After washing the hands, the samples were collected from each volunteer in a separate glass beaker and the collected samples were allowed to grow on nutrient agar media for overnight at 37 C and per ml CFU were calculated. at 3 different randomly selected day(10).

10. Activities Against Skin Pathogens :

MATERIALS AND METHODS :

A. Preparation of Leaf extract and lemon juice:

Tulsi and neem leaves were dried in oven at 50°C and coarsely grinded to get a powder. For methanol extraction, 10g of leaves powder and 90% methanol was added in 250ml flask (maceration) and kept on shaker for 48 hours. After maceration, sample was filtered by whatman no.1 filter paper. This filtrate was placed in water bath at 60°C till thick sticky, dark coloured extract is obtained and lemon were washed, cut into half and squeeze to get a juice. This juice was stored at 4°C for further use.(12)

B. Antibacterial activity of extract against standard organism:

Antibacterial activity of extract and juice was carried out by agar well diffusion method against standard organism. After swabbing culture and adding extract the plates were incubated at 37°C for 24hours. (12)

C. Phytochemical analysis :

Test for carbohydrates (Fehlings test), amino acid (Ninhydrin Test), phenols and tannins, Flavonoids, Saponins (Foam test), Terpenoids, steroids and sterols (Salkowski's test) was performed as per given reference (12)

D. Radical Scavenging Activity by the DPPH Assay:

DPPH assay was performed to check radical scavenging activity of neem extract and lemon juice. Absorbance of all the samples was taken (9). The percentage radical scavenging activity of each extracts was calculated using the following equation

E. Collection of hand isolates from lab assistance:

Hand swabs of lab assistants by using sterile cotton swabs were taken. Plated on nutrient medium and

incubated. Colony characterization, gram staining and biochemical were performed. Isolates were labeled as isolate I, II, and so on and preserved for further use and to study the effect of herbal sanitizer on hand microflora.

$$\text{Percentage radical scavenging activity (RSA \%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100\%}{\text{Absorbance of control}}$$

F. Antibiotic sensitivity test (AST) of pathogens:

To study the susceptibility of bacteria to various antibiotics AST was performed by referring Kirby Bauer and Koneman's chart.

G. MIC determination by Macro agar dilution method :

Minimum inhibitory concentration of all three extracts was done by macro agar dilution method. Four different dilutions of extract using DMSO as diluent was prepared. Undiluted, 1:2, 1:4 and 1:8 were the dilutions used, along with control. 1ml of these dilutions was mixed with 19ml of LB butts and was poured on petri plates. Then culture suspension of 0.5 Mc Farland turbidity was prepared and 5 μ l of culture were spot inoculated on plates. Plates were kept stand for 5mins and then incubated at 37°C for 24 hours. MIC was determined by inhibition of culture at particular dilution. (12)

H. Preparation of sanitizer:

Lemon sanitizer: Add 19ml of 70% Isopropyl alcohol in flask. In that add 2ml Glycerol and 4ml Rose water. Then add 25ml of Lemon juice.

-Lemon sanitizer: Add 19ml 70% Isopropyl alcohol in flask. In that add 2ml Glycerol and 4ml Rose water. Then add 12.5g of neem extract and 12.5ml lemon juice. and 4ml Rose water. Then add 1g of neem extract. (12)

I. Antimicrobial and Antifungal activity of sanitizer:

For this method, Mueller-Hinton agar plates were used. Agar surface of each plate was streaked by a sterile cotton swab with the reference bacterial strain. Then agar plate was punched with a sterile cork borer of 4 mm size and then poured 100 μ L of each sample (sanitizer) with micropipette in the bore. Plates were allowed to stand for 30 min. Then incubated at 37°C for 24 h. Zone of inhibition (mm) of the bacterial strains against three herbal sanitizers was measured(12)

J. Effect of sanitizer on biofilm :

Crystal Violet assay: This is a quantitative method for biofilm detection. Loopful of test organisms was inoculated in a 10 mL of trypticase soy broth with 1% glucose in test tubes. Tubes were incubated at 37°C for 24 h. After incubation, decant tubes was wash with sterile saline. Base of tube was Heat fix. Tubes were then stained with crystal violet (0.1%). Excess stain washed with distilled water. Distained with 4ml of ethanol by incubating for 5mins at room temperature. Recorded reading by taking optical density at 660nm. (12)

K. Efficacy of sanitizer:

To check efficacy a 200mm size of petriplate were taken. In that 200ml of LB agar were poured. After cooling of agar two plates one for control and other for test were selected. Without applying sanitizer hand of assistant were placed on control plate and with applying sanitizer hand of assistant were placed use as test. Then plates were incubated at 37°C for 24hours. The decrease in number of colony was determined. Same procedure was followed for all three sanitizers.

L. Antibiotic potentiation:

This procedure is modification from Kirby-Bauer disc diffusion method. Different dilution of the

sanitizers was prepared ranging from 1:10, 1:20, and 1:30 with sterile distilled water. 1ml of each dilution were added to 9ml of MH agar and then poured into petriplate. MH agar plate without sample was taken as control. Within 15mins after adjusting turbidity (0.5 McFarland standards) of inoculum suspension, a₁₅ sterile cotton swab was dipped into it and was swabbed over entire surface of MH agar medium containing different dilution of sample. The antibiotic disc was placed in agar plate seeded with respective bacteria. Antibiotic disc used were Oxacillin (1mcg) for MRSA and Norfloxacin (10mcg) for Isolate IV. The plates were incubated at 37°C for 24hours. After incubation each inhibition zone diameter (IZD) will be measured and analyzed according to clinical and laboratory standards institute guidelines and the antibacterial activity will be expressed as the mean of IZD (mm)(12)

11. RESULTS :

A. Antimicrobial and Antifungal of leaf extract and lemon juice:

Antimicrobial and antifungal activity of extract against standard organism was performed by Agar well diffusion method. Neem extract was found to be effective against *S. aureus* and MRSA and not against *C. albican* and *P. aeruginosa*. Tulsi extract did not show antibacterial activity against selected organisms. Lemon juice was inhibiting *S. aureus*, MRSA, *P aeruginosa* and not to *C. albican*. Hence neem extract and lemon juice was selected for further study. DMSO was use as diluent for extract which does not show its antibacterial activity against selected organisms (Table 1).(12)

Table 1. Antimicrobial and Antifungal activity of extract by agar well diffusion method

Organism	S.aureus	MRSA	C.albican	P.aeruginos a
	Zone of Inhibition (mm)			
Neem extract	14	10	-	-
Tulsi extract	-	-	-	-
Lemon juice	16	14	-	20
DMSO	-	-	-	-

B. Phytochemical analysis:

Presence and absence of phytochemicals in lemon juice and neem extract as shown in (Table 2).

Table 2. Lemon juice and Neem phytochemicals

Test	Result	
	Lemon juice	Neem extract
Saponins	-	+
Tannin	+	+
Steroid	-	-
Phenol	+	+
Flavonoids	+	+
Carbohydrate	-	-
Amino Acid	-	-
Terpenoid	-	+

Key: +: Present / -: Absent

C. Free radical scavenging assay:

Free radical scavenging assay of neem extract and lemon juice was performed by DPPH method and absorbance was taken at 530nm. Percentage radical scavenging activity was calculated by formula. Neem extract has more radical scavenging activity than lemon juice. Even diluted sample of neem extract and lemon juice shows a radical scavenging activity (Table 3).

Table 3. DPPH assay of neem extract and lemon juice

Concentration	Absorbance at 530nm			
	Neem extract	RSA%	Lemon juice	RSA%
Undiluted	0.28	47.16	0.42	8.69
1:2	0.06	88.67	0.29	36.95
1:4	0.05	90.56	0.28	39.13
1:8	0.01	98.11	0.26	43.47
Control	0.53	-	0.46	-
Blank	0.0	-	0.0	-

E. MIC determination:

MIC for isolate I, IV, VII *aureus* were found to be 1:2. While that for isolate III MIC found to be 1:4. For isolate VI MIC found to be 1:8. No inhibition at culture V, MRSA and *P. aeruginosa* is seen, it means further dilutions were needed to be performed. (Figure 1 a). MIC for isolate I, III, IV, V, VI, VII no growth was seen at any concentration it means further dilutions were needed to be performed. MIC for *S. aureus* and *P. aeruginosa* were found to be 1:2. For MRSA it was found to be at undiluted concentration (Figure 1 b). For *C. albican* no inhibition by neem extract and lemon juice was found at

any concentration.(12)

- **MIC of neem Extract**

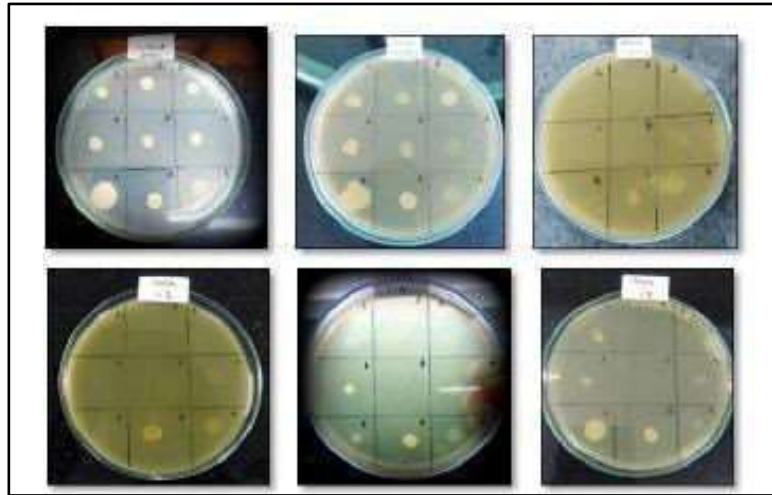


Fig 1:- : MIC of neem extract against

B. MIC of lemon Juice:

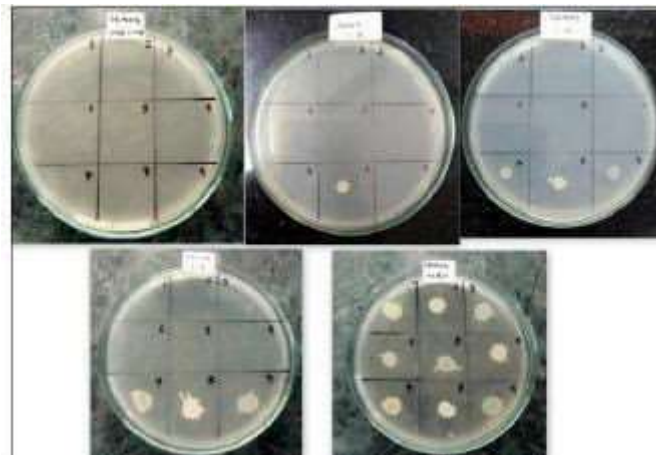


Fig 2:- MIC of lemon juice against selected bacteria was performed by using macro agar dilution method.

F. Sanitizer:

Formulation of sanitizers were done by using neem extract and lemon juice with base iso- propyl alcohol, rose water and glycerol. Three different sanitizers were formulated named as lemon, lemon-neem and neem sanitizer (Figure 2 a,b,c).



a. Lemon b. Lemon-neem c. Neem

Fig 2:- a,b,c: Formulated Sanitizer

G. Antibacterial activity of sanitizer by agar well method:

Antibacterial activity of all formulated sanitizers with positive control that is Sterillium and other control is the base use to formulate sanitizer was checked. Against isolate III and isolate VI no growth on plate was seen. For all other isolates inhibition was seen. It was found that all three sanitizers were showing more inhibition than sterillium and sanitizer base (Figure 3).(12)

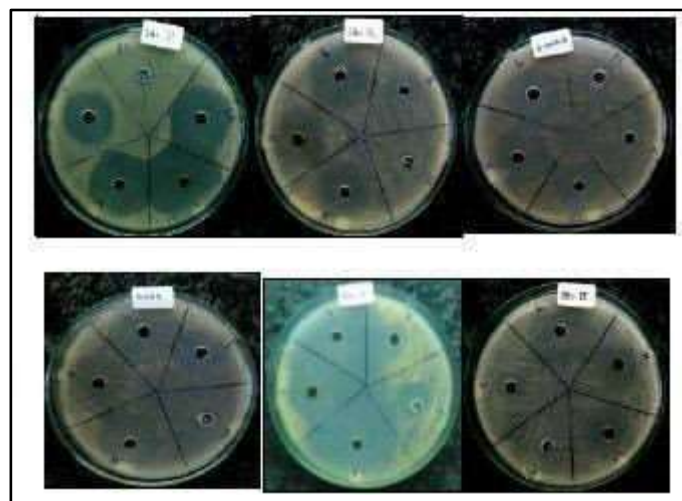


Fig 3:- Antibacterial activity of sanitizers by agar well diffusion method

12. CONCLUSION :

Medicinal Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Plant extracts or secondary metabolites have served as antioxidants in phytotherapeutic medicines to protect against various diseases for centuries Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds. Phytochemicals are secondary metabolites, which are produced by medicinal plant.

In this research Phytochemicals was found to be present in neem extract and lemon juice. Radical scavenging activity was also found to be present in both. MIC concentration of neem extract and lemon juice was checked by using different dilutions against standard culture and hand isolates. Three different

sanitizers were formulated named as lemon, lemon-neem and neem sanitizer. Their antibacterial activity was checked against culture and also compare with sterillium. Formulated sanitizer was found to be more effective than sterillium. Hence it can be use in hospitals. They also showed anti-biofilm activity. Antibiotic potentiation activity of sanitizer against pathogens is changing the sensitivity pattern hence can be use in combination with antibiotic in some formulations.

As more effective is the formulated sanitizer, it can be brought into market. Neem sanitizer due to its bitter taste it cannot be use as hand sanitizer but can be use as floor cleaner, as disinfectant, toilet spray or to clean medical devices as it do not leave any colour traces. Lemon and lemon-neem sanitizer can be use as hand sanitizers. As neem and lemon possesses bioactive molecules it can also be use to formulate cream for wound healing and also for treating other skin diseases. Due to its antibiofilm activity, neem extract and lemon juice both can be use in toothpaste or in mouth wash liquid for treatment of dental plaque.

REFERENCE

1. Larson E. Skin hygiene and infection prevention: More of the same or different approaches? *Clin. Infect. Dis.* 1999; 29: 1287-1294
2. Coronavirus disease 2019 (COVID-19) Situation Report-101. 2020. Available at: https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200430-sitrep-101-covid-19.pdf?sfvrsn=2ba4e093_2. Accessed July 10,2020.
3. Burke JP. Patient safety: infection control-a problem for patient safety. *N Engl. J. Med.* 2003; 348: 651-656.
4. Pittet health-care settings: recommendations of the healthcare infection control practices advisory committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. *Infect Control Hosp Epidemiol.* 2002;23(12 Suppl):S3–40.
5. Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory Committee. Society for Healthcare Epidemiology of America. Association for Professionals in Infection Control. Infectious Diseases Society of America. Hand Hygiene Task Force Guideline for hand hygiene in
6. <https://www.google.com/search?client=firefox-b-m&biw=396&bih=770&tbm=isch&sa=1&q=neem++hand+sanitizer&oq=neem++hand+sanitizer&aqs=mobile-gws-lite..015>
7. Cowan M. M., Plant Products as Antimicrobial Agents. *Clinical Micro. Reviews.* 1999;12(4): 564- 2.D, Allegranzi B, Boyce J. The World Health Organization guidelines on hand hygiene in health care and their consensus recommendations. *Infect Control Hosp Epidemiol.* 2009;30:611– 622. [PubMed] [Google Scholar
8. https://www.researchgate.net/publication/338645442_Formulation_of_an_Herbal_Substitute_f_or_Chemical_Sanitizer_and_its_Evaluation_for_Antimicrobial_Efficiency
9. <https://pharmascope.org/ijrps/article/view/2718/5690> j, zxckjhaldfkzxhfolckhzoc zxmcbzike xcnclnck,ckcchkckzxjpojfadvjasdibfoasfnkllxxjlad kjh
10. https://www.researchgate.net/publication/25326549168_Formulation_Evaluation_and_Antibacterial_Efficiency_of_water-based_herbal_Hand_Sanitizer_Gel
11. Hand Sanitiser- From Wikipedia, the free encyclopedia
12. <https://ijisrt.com/wp-content/uploads/2018/08/Formulation-of-Herbal-Sanitizers-and-Determining-Their-Antimicrobial-Activities-against-Skin-Pathogens.pdf>
13. PalakVyaset al. Antimicrobial Activity of Ayurvedic Hand Sanitizers. *International Journal of*

- Pharmaceutical & Biological Archives. 2011; 2(2):762-766.
14. Larson E. Skin hygiene and infection prevention: More of the same or different approaches? *Clin. Infect. Dis.* 1999; 29: 1287-1294
 15. Burke JP. Patient safety: infection control-a problem for patient safety. *N Engl. J. Med.* 2003; 348: 651-656
 16. Cowan M. M., Plant Products as Antimicrobial Agents. *Clinical Micro. Reviews.* 1999;12(4): 564-82 .. WHO Guidelines on Hand Hygiene in Health Care (Advanced Draft).
 17. MondalSunanda, Kolhapure S.A., Evaluation of the antimicrobial efficacy and safety of Pure Hands herbal hand sanitizer in hand hygiene and on inanimate objects. *The Antiseptic.* 2004;101(3): 112-120.
 18. Widmer AF, Replace hand washing with use of a waterless alcohol hand rub. *Clin Infect Dis* 2000;31:136-43
 19. Berman RE, Knight RA. Evaluation of hand antisepsis. *Arch Environ Health* 1969;18:781-3.
 20. Mackintosh CA, Hoffman PN. An extended model for transfer of micro-organisms via the hands: Differences between organisms and the effect of alcohol disinfection. *J Hyg* 26(Lond) 1984;92:345-55.