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FORMULATION AND EVALUTION OF HERBAL NEEM SANITIZER

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ABSTRACT

The purpose of the study is to maintain a hygienic hand and It is a most important principle for prevention, control, and reduction of healthcare acquired infections. Right method of hand washing and drying can stop the chain of transmission of deadly pathogens from hands to other parts of the body. Hand sanitization aids in nosocomial infections which can be caused by different microorganisms. The purpose of the study was to prepare a herbal hand sanitizer incorporating Azadirachta indica (Neem). Phytochemical analysis of the constituents was executed. The standard culture of microorganisms was used for the analysis of minimum inhibitory concentration of the herbal formulation. The zone of inhibition of the formulated sanitizer and the Sterilium sanitizer was compared. The efficacy of the formulation was tested by using standardized methods (quality control tests). The major aim of the study is to estimate the antibacterial potency of formulated sanitizer.

KEYWORDS: sanitizer, minimum inhibitory concentration, Antibacterial.

INTRODUCTION

The most important aspect of the infection control activity is the hand hygiene. There is an ongoing problem of healthcare associated infections which also includes multidrug resistant infections. Hand hygiene can significantly reduce the risk of cross contamination. Skin is the most exposed part of the body so it can be contagious. The main aim is to prepare and evaluate polyherbal sanitizer from commonly available plants.

There are antiseptic products used to avoid the transmission of skin infection and pathogens. Alcoholic hand sanitizer kills 99% of the bacteria soon after the application.

Nosocomial infections are those which are associated or originated in the hospital or healthcare facility as a result of pathogens.

Escherichia coli, pseudomonas SPP, staphylococcus aureus are common opportunistic microorganisms that primarily cause nosocomial infections. Generally, infection sites are urinary tract, surgical wound, respiratory tract, skin, blood, gastrointestinal tract. Pseudomonas aeruginosa is the most commonly found organism in hospitalized patients. Fungal infections are prominent HIV patients' high frequency of fungal infection is found in candidiasis, aspergillosis, cryptococcosis. Usually, microbes residing in hand is classifies as resident and transient flora.

E COLI - E coli is a bacteria found in the intestine it is also found in the gut of the animals it can cause diarrhea If we eat with contaminated hands. E coli causes disease by releasing a toxin called as **Shiga** thus they are called **Shiga toxin** producing E coli. It can cause life threatening symptoms like fever, bleeding, confusion, seizures.[1]

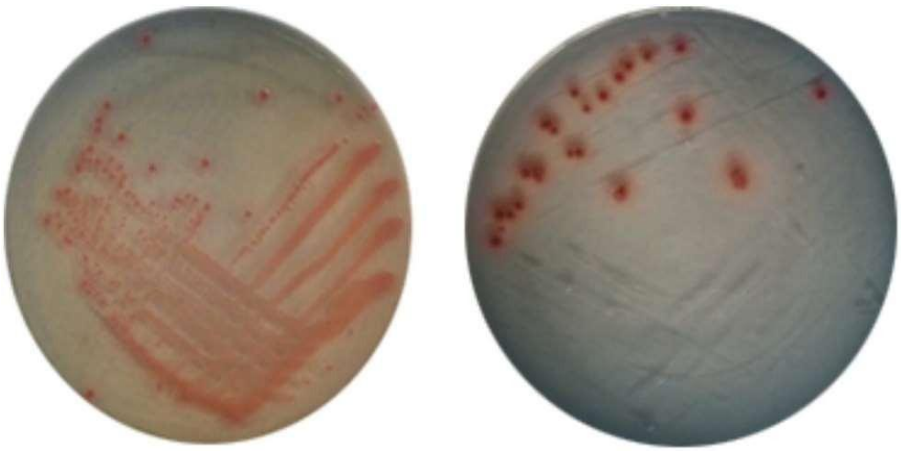

Figure 1. E coli.

PSEUDOMONAS SPP

Pseudomonas is a type of bacteria that is commonly found in water soil and environment. Pseudomonas that causes infection in humans is called as pseudomonas aeruginosa. In 2017 multi drug resistant pseudomonas aeruginosa caused estimated about 600 infections among hospitalized patients and 2700 deaths in the United States this infection can be avoided by keeping the hand clean to avoid spreading germs treating pseudomonas infection has been difficult in recent days due to antibiotic resistance.[1]

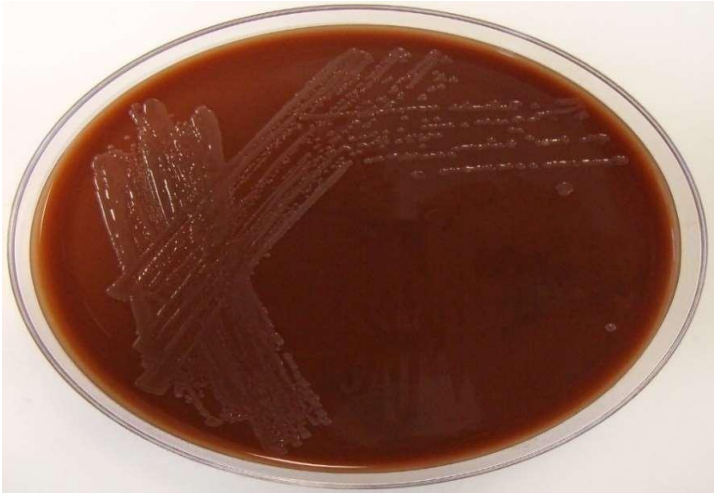

Figure 2. Pseudomonas SPP.

STAPHYLOCOCCUS AUREUS: these bacteria spread by direct contact with the infected person or by using contaminated object. Skin infections are common by the bacteria can spread through the bloodstream and infect distant organs. Skin infections may cause blisters, redness, swelling of infected area staphylococcus is present in nose of about 30% of the healthy individuals and skin of about 20%. The most common staphylococcus infections are the skin infections often causing abscess however the bacteria can travel through the bloodstream and infect almost every part of the body infection of heart valves is called as endocarditis and bone osteomyelitis.[1]

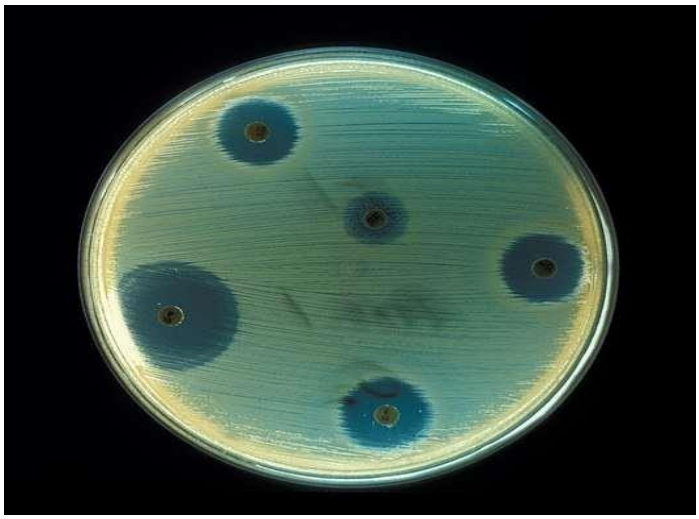

Figure 3. staphylococcus aureus.

Staph toxins – there are many strains of staphylococcus aureus some strains produce toxins that can cause food poisoning, toxic shock syndrome.

Resident flora (EG: Corynebacterium diphtheriae, staphylococcus aureus, staphylococcus epidermis) they colonize into deeper skin layer more resistant to mechanical removal has lower pathogenic potential.

Transient flora – (EG: staphylococcus aureus, gram negative bacilli, candida species. Colonizes on superficial skin layer for short period of time.

In current days consumer prefer readymade formulation or alcohol hand rub rather than the handwashes. Mechanical friction is produced between the hands for a minute followed by rinsing with water then drying thoroughly by disposable towel.

Plants rich in wide variety of secondary metabolites such as tannins, terpenoids, Alkaloids, flavonoids have found antimicrobial properties. While screening found that *Azadirachta indica* (neem) has antimicrobial properties the formulate and evaluate the herbal sanitizer combine the alcoholic extract of neem using suitable excipients which can be used as a readymade herbal sanitizer.[2]

Therapeutic properties of neem: (*Azadirachta indica*)

- Neem is recognized as a medicinal plant well known for its antibacterial, antimalaria, antiviral and antifungal properties. Neem fruit, leaves, stem and bark contain diverse phytochemicals. Some of which were first discovered in Azadirachta seed extracts such as Azadirachta. Used in 1960's as insect antifeedant, growth disruptors and insecticide.
- Medicinal plants will be having diverse range of bioactive molecules, making them rich source of different types of medicines pharmacological studies have accepted the value of plants as potential bioactive molecules.
- Phytochemicals are secondary metabolites which are produced by medicinal plants. So, sanitizer is formulated with complex chemicals as well as herbal materials and their efficacy was checked against the group of microorganisms.
- As neem extract is an important source of compound having antimicrobial, antioxidant, antitumor, antimalarial, antifungal, anti-inflammatory and antiviral properties and antibacterial potential is there in crude extract of different parts (leaves) is checked against clinically significant bacterial strains has been reported.
- Hence in this studies neem leave is been used their antibacterial properties are studied and accordingly sanitizer is formulated.[3]

AIMS AND OBJECTIVES

AIM

To formulate and evaluate a therapeutically effective herbal sanitizer by *using Azadirachta indica*[neem] leaves.

OBJECTIVES

- To formulate and effective herbal sanitizer.
- To maintain hygiene and cleanliness of hands after coming from public places, hospitals, zoo, bird sanctuaries etc.
- To avoid infection spread from hand to different parts of the body which can cause serious health hazards.
- To check the effectiveness of the sanitizer by conducting standardized tests (quality control tests).

NEED OF THE STUDY

- To maintain hygienic hands.
- To avoid hand spread microbial infections.
- To kill pathogens that are spread unexpectedly that can cause health hazards.
- To make hand cleansing process easier and effective.
- To make an affordable low-cost herbal hand sanitizer.

REVIEW OF LITERATURE

1. **Vaibhav Rajendra et al**, stated that the main idea behind formulation of alcohol based herbal hand sanitizer is to sanitize hands. Washing our hands is very much important to reduce, prevent and control the infection. So, this formulation is formulated to maintain hand hygiene. The alcohol-based hand sanitizer claims to kill 99.99% microbes and it is most effective. Different formulations are prepared and characterized using eucalyptus oil as an active pharmaceutical ingredient. Among five formulations, F3 is selected based upon its anti- bacterial activity. Then, F3 formulation is subjected to different evaluation parameters like Odour, Colour, Clarity test, pH and skin irritancy characteristics. The formulation F3 was clear and light orange in Colour with mild Odour. The pH of herbal hand sanitizer found to be 6.5 with no irritation to skin. Anti-microbial properties evaluated showing good Zone of Inhibition in E. coli and S Aureus are 22mm and 26mm, respectively.[4]

2. **Rina Maskare et al,** stated that the main objective of the work was to perform extraction and formulation of herbal hand sanitizer is for "hand hygiene". It is a vital principal in the prevention, control, and reduction of any acquired infection. 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 Soxhlet 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. The higher antimicrobial activity and efficacy of these herbal extracts can be used in the preparation of herbal hand sanitizers on commercial scale.[5]

3. **Rutuja Sunil Patankar et al,** stated that the medicinal properties of plants are been studied since long back and various research on it was carried out and from that various pharmaceutical, health care, cosmetic products are been manufactured. This research was aim to formulate the herbal sanitizer. Sanitizer formulated from neem and lemon juice. Phytochemical and antioxidant properties of neem extract and lemon juice were analyzed. Their MIC was checked against standard culture and hand isolates from lab assistance. MIC of neem extract and lemon juice was found. Three different sanitizers were formulated lemon, lemon-neem and neem sanitizer. Formulated sanitizer was compared with Sterilium sanitizer and it was found that Sterilium was effective against standard organism only and not against hand isolates while formulated sanitizer was effective against both. Even in comparison with respect to zone of inhibition, formulated sanitizers were showing more zones as compared to Sterilium sanitizer. Result obtained showed reduction in bacterial growth after treatment with sanitizer. As neem sanitizer was tested found to be bitter hence instead of using it as hand sanitizer it can be used as a spray on medical devices or as a floor cleaner.[6]

4. **Nand Kishor Wani et al,** told that the Hand hygiene is a vital principle and exercise in the prevention, control, and reduction of healthcare- acquired infections. Right hand washing and drying methods stop the chain of transmission of deadly pathogens (from the contaminated surface/site) form hands to other parts of the body. Hand sanitization is the preeminent aid in preventing nosocomial infections caused by

different opportunistic microorganisms and to get this, the use of hand sanitizer becomes must in recent circumstances.[7]

5. **Fatima Grace et al,** stated that the main aim for the preparation of a herbal hand sanitizer is for "hand hygiene". It is a vital principle in the prevention, control, and reduction of any acquired infection. Mainly hand sanitizer can stop the chain of transmission of microorganisms and other bacteria from hand to different parts of our body. Hand hygiene is important and one of the most critical steps in food production, food service as well as in homes and other day care preparations. Hand sanitizer avoids adverse effects like itching, irritation, dermatitis etc. So, maintaining hand hygiene as the prime criteria-instead of some synthetic formulation, an attempt has been made to formulate an herbal hand wash by using some extracts of commonly available plants like Andrographis paniculata. The formulation was evaluated for its physical parameters. It is sure that these ingredients on combination behave as an effective hand sanitizer.[8]

6. **Jyotsana Singh Chandravanshi et al**, he stated that, hands are the first mode of transmission of microbes and infections. Hand hygiene is a key principle and exercise in the prevention, control and reduction of infections. Due to COVID pandemic, the need of hand sanitizer has increased which causes less dryness to hand. Considering the need, we prepared a herbal sanitizer using seven plant extracts with other ingredients including isopropyl alcohol, camphor, hydrogen peroxide, glycerol and water. The ingredients were selected on the basis of their antimicrobial property. The ingredients and sanitizer were evaluated for antimicrobial property and showed potent activity against gram-positive bacteria S. aureus, whereas mixture of extracts showed potent activity against all the bacterial strains. The antimicrobial activity was compared with other commercial hand sanitizers and maximum activity was showed against Klebsiella spp. and minimum against E. coli and P. aeruginosa by all the sanitizer used. The efficacy of hand sanitizer was checked on hands of 38 volunteers of laboratory workers, patients and their relatives with written and oral consent. The sanitizer reduced or eliminated the growth of pathogens isolated from hands. Time interval effect was also checked at a time gap of two hours also showed the effect of hand sanitizer for longer time with reduction in bacterial growth. The sanitizer pH was alkaline with good shelf, texture and odor. No turbidity was seen when kept at higher

temperature for 3 months and showed no skin dryness with soothing effect after using sanitizer on different volunteers.[1]

7. **Adenilton C da Silva et al**, stated that the use of alcohol-based hand sanitizers is recommended as one of several strategies to minimize contamination and spread of the COVID-19 disease. Current reports suggest that the virucidal potential of ethanol occurs at concentrations close to 70%. Traditional methods of verifying the ethanol concentration in such products invite potential errors due to the viscosity of chemical components or may be prohibitively expensive to undertake in large demand. Near infrared (NIR) spectroscopy and chemometrics have already been used for the determination of ethanol in other matrices and present an alternative fast and reliable approach to quality control of alcohol-based hand sanitizers. In this study, a portable NIR spectrometer combined with classification chemometric tools, i.e., partial least square discriminant analysis (PLS–DA) and linear discriminant analysis with successive algorithm projection (SPA–LDA) were used to construct models to identify conforming and non-conforming commercial and laboratory synthesized hand sanitizer samples. Principal component analysis (PCA) was applied in an exploratory data study. Three principal components accounted for 99% of data variance and demonstrate clustering of conforming and non-conforming samples. The PLS–DA and SPA–LDA classification models presented 77 and 100% of accuracy in cross/internal validation respectively and 100% of accuracy in the classification of test samples. A total of 43% commercial samples evaluated using the PLS– DA and SPA–LDA presented ethanol content non-conforming for hand sanitizer gel. These results indicate that use of NIR spectroscopy and chemometrics is a promising strategy, yielding a method that is fast, portable, and reliable for discrimination of alcohol-based hand sanitizers with respect to conforming and non-conforming ethanol concentrations.[9]

8. **Booq, R.Y et al,** stated that hygiene is an essential factor to prevent or minimize the spread of infections. The ability to prepare an alcohol-free hand sanitizer (AFHS) with antimicrobial properties is crucial, especially during pandemics, when there are high demands and a low supply chain for ethanol and isopropanol. The objective of this study was to prepare AFHS gels based on natural materials that contain essential oils (EOs) that would be effective against a broad spectrum of pathogens. The results showed that the organoleptic characteristics of all prepared hand sanitizer gels were

considered acceptable.[10]

9. **A Chakraborty et al,** he stated that novel Corona Virus has spread to 188 countries around the world which made the people infected, facing moderate respiratory illness. Currently one of the major strategies to deal with COVID-19 and reduce community transmission of infections is the frequent use of hand sanitizers. However, a large section of common mass is unable to buy them due to higher price. Therefore, an approach has been presented here to produce cheaper sanitizers with easily available herbal ingredients like Aloe Vera gel, boiled water, surgical spirit, Glycerin etc. The estimated making cost of 100 ml of sanitizer was 16 rupees. The mass production of this sanitizer can be very effective for large scale use of sanitizers by common people.[11]

10. **Firdous Shaikh et al,** stated that this research paper is centered on the effectiveness, bringing to light an optimistic effect of herbal hand sanitizer using neem leaves extract. A large portion of the research has focused on hygiene by controlling the entry of pathogens into the body through hands. Having run over the positive advantages on reducing the microbes, the aim for the current study is set up. Natural herbal hand sanitizers are effective, environment friendly, and biodegradable, inexpensive.[12]

11. **Alibha Rawat et al,** stated that hand sanitizer is an alternative to the hand washing with soap and water. Hand sanitizer is the most important measure to avoid the transmission of harmful microbes and prevent the infections, to keep the skin safe from harmful microorganisms and to prevent spreading of many infectious diseases. Hand sanitizers are available in the form of liquid, foam or easy flowing gel formulation. Both alcohol and alcohol-free hand sanitizers are available in the market. But the regular use of alcoholic sanitizer destroys your hand skin; therefore, there is a need to prepare alcohol-free sanitizer and herbal hand sanitizer which is mild to skin and effective at killing germs. Plants are rich source of vast variety of secondary metabolites such as tannins, terpenoids, alkaloids and flavonoids etc. and which have been found to possess antimicrobial properties. The current review work attempts to summarize the work of researchers in India and worldwide who are engaged in the studies of herbs as potential antimicrobials for the development of herbal hand sanitizer.[13]

12. **Dr. R. Kalaivani et al,** she stated that an ever-increasing number of customers might want good hygiene is a minister to health, comfort, and social interactions. Current society is well aware of hygiene. Washing hands is one of the simplest, most effective ways to get rid of germs and avoid infection. Transient microorganisms can be of any type, from any source may contact the body. As hands carry out the majority of functions of the human's body and are exposed to a variety of substances, hands hygiene plays a chief conscientiousness. Herbal plants are the unique resources of every country with potent medicinal properties pave ways towards sustainable health.[14]

13. **Sajid A Mulani1 et al,** he stated that hand hygiene is now creating more awareness in the people due to pandemic COVID -19. It plays important role in the prevention, control and reduction of any acquired infection. This can stop the chain of transmission of microorganism and other bacteria from hand to different parts of our body. Herbal medicines have been extensively utilized as effectual remedies for the prevention and management of multiple health conditions. The present research was carried out to formulate and evaluate the herbal hand sanitizer using extract. The formulation was evaluated for its physical parameters. It is sure that the combination of ingredients behaves as an effective hand sanitizer.[15]

14. **Muhammad Mujiburohman et al,** he stated that due to in situations the pandemic of the corona virus disease 2019 (COVID-19), World Health Organization (WHO) has addressed the importance of hand hygiene in avoiding the spread of the COVID-19 virus. Normally hand hygiene facilities is included alcohol-based hand sanitizer. Nevertheless, high dose of alcohol or high frequency of alcohol-based hand sanitizer application increase skin dryness causing skin burning exacerbates high risk of skin infection. Therefore, natural bioactive compound derived from the available agricultural crop was chosen to be developed into the safe and efficient alcohol-based hand sanitizer accordingly. Alkaloids are said to be abundant in the bark, stems, leaves, and seeds. Leaves and stems have saponin and tannin; gum is present in the trunk.[16]

15. **N Uma Maheswari et al,** she stated that hand hygiene is a vital principle and exercise in the prevention, control and reduction of healthcare acquired infections. A practice of hand sanitizing is very important to eliminate the microbial contamination

especially work in laboratories, hospital and even at home. Proper use of hand sanitizer reduces the transmission of infection also. Herbal hand sanitizer was prepared by using herbs Neem.[17]

MATERIALS AND METHODS

MATERIALS

The herbal sanitizer was prepared by the following ingredients given below

- *Azadirachta indica* (Neem)
- Hydrogen peroxide
- Glycerol
- Ethanol
- Distilled water
- Other common excipients are procured from standard company sources.

Table 1: Ingredients used in formulation.

Sl no	Ingredients	Role of ingredients
1	Neem	Herbal drug
2	Hydrogen peroxide	Disinfectant
3	Glycerol	Humectant, Emollient, and Lubricant
4	Ethanol	Anti-infective
5	Distilled water	Vehicle

METHODOLOGY

Formulation of Herbal Neem Sanitizer:

- 10g plant extract of powdered leaves were extracted successively with 100 ml of ethanol at 40-50°C in Soxhlet extractor until the extract was clear.

Figure 4. sample taken from the market.

- The extracts were evaporated to dryness and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.
- After uniform mixing, Neem was added with slow stirring to avoid formation of possible air bubbles in the product and kept aside for 24 hrs.

Figure 5. Neem powder used.

- *Azadirachta indica* extracts added to denatured alcohol along with Glycerol, Hydrogen peroxide was mixed with aqueous phase.
- Finally, Propyl Paraben was added as preservative and rose oil as perfume.
- And mixed with slow stirring to obtain uniform product.
- Prepared product was stored in air tight containers.[18]

Figure 6. Soxhlet extractor.

EVALUATION STUDIES

1. **Appearance**

i. Odour: It was determined manually.

ii. Color: It was determined visually.

2. **Determination of pH:** The herbal hand sanitizer's pH was measured with a digital pH meter.

Procedure for pH measurement

- calibration of pH meter was conducted by using buffer solution of pH 7.00, 4.00, 9.20 and the performance of pH electrode was measured.
- Using process of the pH meter may show a little deviation in the operating procedure depending on pH meter used but the process is similar.
- Electrode and the temperature probe was cleaned using deionized water and gently wiped with soft tissue paper.
- The electrode was dipped in the solution (sanitizer).
- The pH mode key is pressed which is present on the board.
- pH was displayed on the instrument.
- Reading was saved if required print the reading by using printer.
- The electrode and the temperature probe was again cleaned with distill water wiped gently with tissue paper and at last reset the instrument.[19]

Figure 7. pH meter.

3. **Clarity test:** A test for clarity was determined to evaluate presence of visualized particulate matter.

Procedure for Clarity test

Particle tests were carried out by observing the liquid hand sanitizers whether there were deposits or particles in the products. The test was carried out by looking at the presence or absence of black and white background.[20]

4. **Skin Irritation Test:** Skin Irritancy of hand sanitizer was evaluated by taking small amount of formulation on palm. Checked for local irritation or any inflammatory reactions (if present or not).
5. **Antibacterial activity of hand sanitizer:** The Antibacterial activity of hand sanitizer was evaluated by disc diffusion method using Agar media.

Procedure for Antibacterial assay

- Antibacterial activity of different compounds was tested by agar well diffusion assay (Zhang et al., 2009) with some modifications.
- Petri dishes were prepared by pouring 20 ml of sterilized NA media under aseptic condition and allowed to solidify.
- After solidification of the media, 100 µl of standardized test microbial inoculums of Gram-positive bacteria S. aureus (MTCC 7443) Gram-negative bacteria and E. coli (MTCC-7410) were spreaded uniformly using sterile cotton swabs.
- 6 mm diameter agar is drawn from plate to form a well using sterile cork borer. Antibiotic gentamycin was used as positive control, DMSO as negative control.
- After keeping at 4 ºC for 4 hours for the diffusion of antibacterial metabolites, thereafter plates were incubated at 37 ºC for 24 h.
- The diameter of the inhibition zone around the well is measured in millimeter (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity.[21]

Figure 8.Antibacterial testing in petridish.

RESULTS AND DISCUSSION

1. Test for color and Odour

Color- The color was checked by normal observation through naked eyes and color was found to be the light green.

Odour- The Odour was found to be Pleasant and Appealing because of the usage of Rose water as a Perfuming agent. It intends the user to use the sanitizer without any hesitation.

2. pH test

The pH test of the sanitizer was measured by using a calibrated pH meter and the pH of the solution was found to be 7.06

Table 2: pH values.

Standard pH	Obtained pH
Above 7	7.06

Figure 9. determination of pH.

3. Clarity test

The sanitizer container was held against white and the black backgrounds to check the presence of any particulate matter or any powder particles of neem.

No particle was found in the sanitizer the sanitizer was found to be fully clear and homogeneous solution.

Figure 10 clarity test.

4. Skin irritation test

Sanitizer was put on 5 students of 8th Sem B. Pharm and it was found that none of the students experienced irritation, redness, swelling or any unwanted/unintended reactions. The experience of using the sanitizer was good as told by the students who used it.

Figure 11 Skin Irritation Test.

5. Antibacterial test / antibacterial assay

Antibacterial assay was done by using standard protocol. It was done by Alekhya agro solutions (A private institute) the sanitizer was found to show antibacterial activity against the organisms E. coli and Staphylococcus aureus, the zone of the inhibition of the bacteria was found to increase with the increase in the concentration.

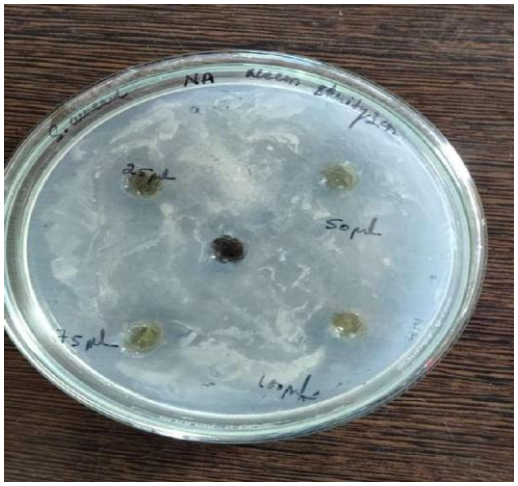

Figure 12 Growth of S aureus Bacteria.

Figure 13 Growth of E coli Bacteria.

The sanitizer was tested on the bacterial colonies in the volume of microliters (µl), 25µl, 50 µl, 75 µl, and 100 µl volume of the sample was tested against microorganism the standard antibiotic gentamycin is used as positive control and DMSO as a negative control. 25 µl of the sanitizer was found to know zone of inhibition of 6mm on both organisms, 50µl of sanitizer was found to know 9mm on both organisms, 75 µl of sample was found to know 10mm of zone of inhibition, 100 µl showed 11mm zone of inhibition, gentamycin antibiotic showed 14mm of zone of inhibition on S. aureus and 15mm on E.

coli, so it can be concluded that sanitizer has good antibacterial activity against both organisms.

Table 3: Results of Antibacterial Assay.

Sl no	Name	S. Aureus	E. coli
1.	25 µl	6mm	6mm
2.	50 µl	9mm	9mm
3.	75 µl	10mm	10mm
4.	100 µl	11mm	11mm
5.	Gentamycin	14mm	15mm

CONCLUSION

Medicinal plants provide wide range of bioactive molecules making them rich source of medicines.

Secondary metabolites have served as phytomedicinal ingredients for many years. Phytochemical are secondary metabolites which are produced by the medicinal plants. As discussed, phytochemical is present in the neem plant.

Minimum inhibitory concentrations of neem sanitizer were found and checked against the standard culture of E coli and S aureus in different dilutions. The sanitizer was found to be effective hence it can be used in hospital.

pH test, Antibacterial assay, Skin irritation test, Clarity test was conducted and sanitizer passes all the tests.

It can also be concluded that herbal sanitizer has significant antimicrobial effect on the specified organisms E coli and Staphylococcus aureus. Thus, there is immense potential in establishing the use of antimicrobial herbal products as a measure to control multi drug resistant microbes as well as check their spread through hands.

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FUTURE STUDIES

1. The study was conducted on only two organisms. There are too many organisms that could cause nosocomial infection.
2. The two organisms E coli and Staphylococcus aureus does not represent whole population of microbes thus the sanitizer cannot prevent all kinds of infections.
3. The sanitizer can show altered or different results when formulated with different substitutes of excipients, so it cannot be said that it is not 100% efficient sanitizer.
4. Skin irritation test was done only on few students so it cannot conclude that it cannot cause irritation when we used by large populations of people, non-irritancy cannot be 100% assured.

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ANNEXURES

ALEKHYA AGRO SOLUTIONS

GSTIN : 29KJZPS0005P1Z3

fssai Lic.No.21221130000904

AL/22/2022-23 Date: 17/09/2022

Sub: Evaluation of herbal sanitizer:

Sample submitted by Participants: 100 ml of sample

1. Nachikethana C. R.
2. Rakshith M. L.
3. Rahul K. S.
4. Sanjay B

(8th Semester, B Pharm students, Sarada Vilas college of Pharmacy)

All above are working under the guidance of

Siddartha H. N.
Assistant Professor
Department of Pharmaceutics
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Report :
Antibacterial assay :
Material and Methods:

Antibacterial activity of different compounds was tested by agar well diffusion assay (Zhang *et al.*, 2009) with some modifications. Petridishes were prepared by pouring 20 ml of sterilized NA media under aseptic condition and allowed to solidify. After solidification of the media, 100 µl of standardized test microbial inoculums of Gram positive bacteria *S. aureus* (MTCC 7443) Gram-negative bacteria and *E. coli* (MTCC-7410) were spreaded uniformly using sterile cotton swabs. 6 mm diameter agar is drawn from plate to form a well using sterile cork borer. Antibiotic gentamycin was used as positive control, DMSO as negative control.

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Yelwala - 571 113, Mysuru.

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After keeping at 4 ℃ for 4 hours for the diffusion of antibacterial metabolites, thereafter plates were incubated at 37 ℃ for 24 h. The diameter of the inhibition zone around the well is measured in millimeter (mm) and the average of three repeated agar discs were taken to assess the strength of antibacterial activity.

Observation:

Sl. No.	Name	*S. aureus*	*E. coli*
1.	25 µl	6 mm	6 mm
2	50 µl	9 mm	9 mm
3	75 µl	10 mm	10 mm
4.	100 µl	11 mm	11 mm
	Gentamycin	14 mm	15 mm

Report: The given **sample (Sanitizer)** has antibacterial activity against ***S. aureus*** **and** ***E. coli***

Dr. ₹ 14/9/22

Alekhya Agro Solutions
24, Behind RMP, Rajadhani 2
Yelwala - 571 113, Mysuru

Definitions

1. Infection

An infection is the invasion of tissues by pathogens. Their multiplication and reaction of the host with tissues to the infections agent and the toxins they produce. An infectious disease also known as transmissible disease or communicable disease is an illness resulting from the infection.

2. Health hazard

Health hazards are chemical, physical or biological factors in our environment that can have negative impose in our short- or long-term health. Expose can occur through touch, inhalation, and ingestion. Understanding the risks of these hazards can help us to take action to avoid or mitigate these risks.

3. Transmission

The process of passing of infection from 1 person to another person.

4. Pathogen

A pathogen is defined as an organism causing disease to its host, with the severity of the disease symptoms referred to as virulence. Pathogens are taxonomically widely disease and comprise viruses and bacteria as well as unicellular and multicellular eukaryotes.

5. Nosocomial infections

Nosocomial infections also referred to as healthcare also referred as healthcare associated infections (HAI) are infection acquired during the process of revolving healthcare that was not present during the time of admission.

6. Phytochemical

Phytochemicals can be defined as in the strictest sense to as chemicals produced by plants. However, the term is generally used to describe chemicals from plants that may affect health but are not external nutrients.

7. Minimum Inhibitory Concentration (MIT)

Minimum inhibitory concentrations are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganism after overnight incubation and Minimum bactericidal concentrations (MBCS) as the lowest concentration of antimicrobial that will prevent the growth of an organism.

8. Antibacterial

Antibacterial usually refers to an antibiotic, a principle type of antimicrobial agent used mainly against bacteria it may kill or inhibit them.

9. Hygiene

According to WHO (World Health Organization) hygiene refers to the conditions and practices that need to maintain the health and prevent the spread of diseases.

10. Multi Drug Resistant Infection

Multi Drug Resistant (MDR) bacteria are one of the most important current threats to public health typically MRR bacteria are associated with Nosocomial infections.

11. Cross Contamination

Cross Contamination is the physical movements or transfer of harmful bacteria from one person or place to another.

12. Contagious

Contagious disease is an infectious disease that is readily spread by transmission of pathogens through contact (Direct or Indirect) with in infected person.

13. Antiseptic

An antiseptic is an antimicrobial substance or compound that is applied to living these within to reduce the possibility of infection, sepsis or putrefaction. Antiseptics are generally distinguished from antibiotics by the latter's ability to safety destroy bacteria within the body and from disinfectants which destroy microorganisms found on living objects.

14. Candidiasis

Candidiasis is a fungal infection due to any type of candida (A type of Yeast) signs and symptoms include white patches on the tongue or other areas of tongue and mouth. Other symptoms include soreness and problem in swallowing.

15. Aspergillus

Aspergillus is an infection caused by aspergillus a common mold (A type of fungus). Less often infection can develop when spores enter the body through cut or wound.

16. Cryptococcosis

Cryptococcosis is an infectious disease with worldwide distribution and wide array of clinical presentations caused by pathogenic encapsulated yeast. Currently there are 2 species of cryptococcus that commonly cause disease in humans, Cryptococcus Neoformans / Cryptococcus Gatii.

17. Resident Flora

The Resident Flora consists of microorganisms residing under the superficial cells of Stratum Corneum and can also be found on the surface of the skin.

18. Transient Flora

Transient Flora which colonizes the superficial layers of the skin, is more amendable to removal by routine hand hygiene. Transient microorganisms do not usually multiply on the skin but they survive and radically multiply on the skin surface.

19. Diarrhea

Diarrhea is passing looser or more frequent stools than is normal for you. It affects most people from time to time and is usually nothing to worry about. However, it can be distressing and unpleasant until it passes which normally takes few days or weeks.

20. Antibiotic Resistance

Antibiotic Resistance happen within the germs and longer response to antibiotics designed to kill them.

21. Abcess

An Abcess is a painful connection of fus, usually caused by the bacterial infection. Abcess can develop anywhere in the body.

22. Endocarditis

Endocarditis is a life-threatening inflammation of the inner lining of heart's chamber and valves. Endocarditis is usually caused by an infection. Bacteria, Fungi or other germs get into the bloodstream and attach to damaged areas in the heart.

23. Osteomyelitis

Osteomyelitis is an infection of bone. Infections can reach bone by travelling through the bloodstream. Infections can also begin in the bone itself or the injury exposes the bone to

germs.

24. Formulation

A formulation is a mixture of ingredients prepared in a certain way and used for specific purpose.

25. Tannins

Tannins (Tannic acid) are water soluble polyphenols that are present in many plant foods. They have been reported to be responsible for intake, growth, rate, feed efficiency, net metabolizable energy and protein digestibly in experimental animals.

26. Terpenoids

The Terpenoids are a large and naturally occurring organic chemicals derived from the 5-carbon compound isoprene polymers called terpenoids.

27. Alkaloids

An Alkaloid is a class of naturally occurring organic Nitrogen-containing compounds that are frequently found in plant kingdom.

28. Flavonoids

Flavonoids is a group of natural substances with variable phenolic structures are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for beneficial effects on health and efforts are being made to isolate the ingredients called flavonoids.

29. Antibacterial

Antibacterial usually refers to an antibiotic, a principle type of antimicrobial agent used mainly against bacteria it may kill or inhibit them.

30. Antimalarial

Antimalarial drugs are used for the treatment and prevention of Malarial infection.

31. Antiviral

Antiviral medications help your body fight of viruses that cause disease. They can reduce the symptoms of viral infections and shorten the length of illness.

32. Antifungal

Antifungals are the drugs used to treat fungal infections in hair, skin and nails.

33. Bioactive molecules

Bioactive compounds have actions in the body that may promote good health. They are being studied in the prevention of cancer, health disease and other diseases, examples of bioactive compounds include., lycopene, resveratrol, lignan, tannins and indoles.

34. Soxhlet Extractor

A Soxhlet Extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet. It was originally designed for the extraction of lipid from solid material. Soxhlet extractor is used when desire compound has a limited solubility in a solvent the impurities insoluble in solvent.

35. Preservative

A Preservative is a substance or a chemical that is added to products such as Food products, Beverages, Pharmaceutical Drugs, Paints, many other products to prevent decomposition bt microbial growth or by undesirable chemical changes.

36. Perfume

Perfume is a mixture of fragrant essential oils or aroma compounds, fixatives and solvents usually in liquid form, used to give the pharmaceutical a good smell.

37. pH

pH denotes potential of Hydrogen is a scale used to specify the acidity or basicity of an aqueous solutions. Acidic solutions (solution with higher concentration of H+ ions) are measured to have lower pH values than basic or alkaline solutions.

38. Shelf Life

The period of time during which a material may be stored and remain suitable for use broadly. The period of time during which something remains popular.

39. Sterilium

Sterilium is suitable for hygienic and surgical rub in hand disinfection. Sterilium is used as ready to use alcohol-based rub in product.

40. Sanitizer

Hand Sanitizer is a liquid, gel, foam generally used to kill many viruses/bacteria/microorganisms on hands.

41. Secondary Metabolites

Secondary metabolites are substances manufactured by plants that make them competitive in their own environment. These small molecules exert a wide range of effects on the plant itself and on other living organisms.