



## Preparation and evaluation of herbal sanitizer by using pomegranate peel and lemon seed

*Dr.R.Sivakumar<sup>a\*</sup>, M.Aruna<sup>b</sup>, S.Harshini<sup>c</sup>, A.V.Anju<sup>d</sup>*

<sup>a</sup>principal, Paavai college of pharmacy and research, Namakkal, India

<sup>bcd</sup>Assistant Professor, Department of pharmaceutics, Paavai college of pharmacy and research, Namakkal, India

### ABSTRACT :

Amidst growing concerns regarding the effectiveness and safety of conventional sanitizers, there is a burgeoning interest in herbal alternatives. This study focuses on the preparation and evaluation of a novel herbal sanitizer formulated from pomegranate peel and lemon seed extracts. Pomegranate peel and lemon seed, renowned for their antimicrobial properties, were chosen as the primary ingredients. The extraction process involved the use of suitable solvents to obtain potent bioactive compounds. The formulated sanitizer was subjected to comprehensive evaluation to assess its antimicrobial efficacy against a spectrum of pathogens commonly encountered in daily life. Additionally, its stability, skin compatibility, and scent were evaluated to ensure its practicality and acceptability. Preliminary results demonstrate promising antimicrobial activity against both gram-positive and gram-negative bacteria, as well as fungi. Furthermore, the herbal sanitizer exhibited good stability and skin compatibility, making it a potentially viable alternative to conventional sanitizers. Further optimization and clinical studies are warranted to explore its full potential and efficacy in real-world settings. This research underscores the importance of harnessing natural sources to develop effective and sustainable sanitization solutions.

Keywords: Herbal sanitizer , Pomegranate peel, Lemon seed, Natural antimicrobial agents, Extraction methods, Phytochemical analysis

### Introduction

Hand hygiene is now regarded as one of the most important element of infection control activities. In the wake of the growing burden of health care associated infections, the increasing severity of illness and complexity of treatment, superimposed by multi-drug resistant pathogen infections, health care practitioners are reversing back to the basics of infection preventions by simple measures like hand hygiene. This is because enough scientific evidence supports the observation that if properly implemented, hand hygiene alone can significantly reduce the risk of cross-transmission of infection in healthcare facilities. 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. The bacteria resides on hands are classified in two categories namely resident or transient. The resident flora are resides under the stratum corneum and can be found on surface of skin, namely *Staphylococcus epidermis*, *S. hominis*, *Corynebacteria*, *Propionibacteria*, *Dermobacteria*, *Micrococci* and *fungi Malassezia*. The resident flora protects skin and has antagonistic functions, but cause infections in sterile body cavities, eyes or on non intact skin. Transient flora colonizes the superficial layers of the skin and gets removed by routine hand hygiene, these flora depends on individual profession, habit and skin moisture and sporadically multiply on skin surface. The hands on healthcare workers get colonize while handling patients include pathogenic flora such as *S. aureus*, *Enterococcus spp*, *acetobacter* , *Staphylococcus aureus (MRSA)* yeast and many more. These can be source of nosocomial infections if hand hygiene not maintained. These pathogens cannot be removed by simple washing, therefore hand sanitizing is required. Nowadays, in COVID-19 pandemic the need of cleaning hands has become mandatory and people have become aware of cleaning hands. Hand sanitizer or hand antiseptic is an alternative to the hand washing with soap and water

In the wake of the global health challenges posed by infectious diseases, the importance of effective sanitization practices has been underscored. Traditional sanitizers often contain chemicals that may raise concerns regarding their long-term effects on health and the environment. As a result, there has been a growing interest in exploring natural alternatives derived from botanical sources. Pomegranate peel and lemon seed are two such botanicals renowned for their antimicrobial properties and potential applications in sanitization. Pomegranate (*Punica granatum*) peel, a byproduct of the fruit processing industry, is rich in bioactive compounds such as polyphenols and flavonoids. These compounds exhibit notable antibacterial and antifungal properties, making pomegranate peel an attractive candidate for antimicrobial formulations. Similarly, lemon seeds (*Citrus limon*) are known for their high content of essential oils, particularly limonene, which possesses potent antimicrobial activity against a broad spectrum of pathogens.

This study aims to explore the feasibility of utilizing pomegranate peel and lemon seed extracts in the formulation of a herbal sanitizer. The process involves extracting bioactive compounds from both botanical sources and incorporating them into a sanitizing solution. The effectiveness of the herbal sanitizer will be evaluated through comprehensive microbiological assays to assess its antimicrobial activity against common pathogens, including bacteria and fungi.

Furthermore, considerations will be made regarding the stability, sensory attributes, and safety profile of the herbal sanitizer. By harnessing the natural antimicrobial properties of pomegranate peel and lemon seed, this research endeavors to develop a sustainable and eco-friendly alternative to

conventional sanitizers. If successful, the herbal sanitizer could offer a promising solution for promoting hygiene practices while minimizing the environmental impact associated with chemical-based sanitization products. In summary, this study represents a step towards the development of a novel herbal sanitizer formulation, capitalizing on the antimicrobial potential of pomegranate peel and lemon seed extracts. The findings of this research could have implications for public health, environmental sustainability, and the utilization of agricultural byproducts in value-added applications.

Every fruit have their own antibacterial or antimicrobial compounds which have a role in inhibiting bacterial or microbial growth. The limonoids which are present in the Citrus species such as Citrus lemon has the ability to inhibit the bacterial activity. On top of that, flavonoid and essential oil are one of the plenty nutrients found in the lemon (C. lemon) peel that can be consumed for its 20 antimicrobial and anticancer activity. Therefore, an analysis on the antimicrobial activity of ethanolic extract of the C. lemon peel of 25%, 50%, 75% and 100% have been conducted against E. coli using the disc diffusion method which results in 15.03 mm, 16.17 mm, 15.83 mm, 18.77 mm of the average inhibition zone respectively, thus summarize that those four different percentages of ethanolic extracts have strong antimicrobial activity against E. coli. The extraction of citrus fruit juice is typically the main product required in the country's processing industry. Hence, the citrus fruit waste is left as waste products like peel, seeds and pulps. Thus, the peel extracts of lemon and orange have been used in the enquiries to evaluate their antibacterial activities. The findings show that lemon and orange peel extracts have the best antibacterial activity against B. cereus in methanol and ethyl acetate, resulting in a MIC value to be equal to MBC value (31.25 µg/mL).

Lemon is one of the citrus fruits in which its peel is highly recognized to have flavonoids, which are good antioxidative agents, essential oil and also other functional compounds. The presence of alkaloids, saponin, flavonoids, carbohydrates, glycosides, citric acids and tannins in lemon by carrying out the phytochemical analysis on methanolic extract of the dried C. lemon. Besides, the citrus olive oil has been classified as nutraceuticals because of the existence of carotenoids, naringenin and minor phenolics as their functional compounds. Nutraceutical is defined as a nutrient derived from food or part of food that provides functional advantage or protection against prolonged disease. On the other hand, the valuable peel of orange which contains hesperetin, naringenin, apigenin, neohesperidin, hesperidin, and naringin as their main phytoconstituent flavonoid, as well as hydroxycinnamic acids (HCA), such as p-coumaric acid, caffeic acid, and ferulic acid 18 as their major phenolic compounds can be greatly industrialized into good value added products despite its low-priced resources. Citrus bagasse, which is mostly composed of peel, comprises essential oils and phenolic compounds that have a variety of biological functions. The process of drying citrus fruit peel could affect the degree of bioactive compounds such as ascorbic acid, β-carotene, and flavonoids in ripened and fresh orange peel (Citrus valencia and Citrus balady) and tangerine (Citrus reticulata). Depending on the species, there is a contrasting composition of hydrocarbons, sesquiterpenes, alcohols, aldehydes, esters, and even other oxygenated citrus peel extract derivatives

---

## Literature Review

G. Karthikeyan and A.K. Vidya, performed phytochemical screening, antioxidant efficacy testing and antibacterial activity of Pomegranate peel. Different extracts (Aqueous, Acetone, ethanol and Hexane) found to contain phytochemicals such as phenols and flavonoids that is present in highest amount in inedible part of the fruit. It was found that acetone extract of Pomegranate peel has the highest DPPH activity; on the contrary, ethanol extract showed paramount flavonoids activity, total phenolic activity and FRAP activity. Pomegranate peel was concluded to have both antioxidant and antibacterial activity as well. Arshad Husain Rahmani et al., investigated that the different parts of Pomegranate viz. fruits, seeds, peel and leaves can cure diseases. Pomegranate consumption is safe and devoid of side effects as confirmed by animal models and clinical trials. Priyanka Kesur et al (2016) performed phytochemical analysis of Pomegranate peel and juice extract. Peel extract showed the presence of Alkaloids, Saponins and free amino acids. Juice extract showed the presence of Glycosides, 21 proteins, Vitamin C and free amino acids. Glycosides, Flavonoids, Tannins, free amino acids and Carbohydrates were present in Methanol : chloroform extract. Methanolic and Methanol : Chloroform extracts of juice and peel of Punica granatum showed highest Zone of Inhibition, showed 45% and 25% antioxidant activity respectively. 63% Hydrogen peroxide scavenging activity is also present which was found using ascorbic acid as a standard. Punica granatum was found to be effective against B. cereus, B. megaterium P. vulgaris and P. aeruginosa at 12,500 µg/ml and for B. subtilis, S. typhi, S. typhi A, S. typhi B 25,000 µg/ml was found to be effective. Aspergillus Niger found more sensitive than Rhizopus oryzae.

Alessandra Masci et al., extracted whole Pomegranate fruit blend. It was found that peel is rich in polyphenols, flavonoids, punicalagin and ellagic acid. High phenolic content was found to concentrate in ethyl acetate. Ellagic acid was found to be dominant in Soxhlet extract of pomegranate peels. The study shows that a strong correlation exist between total phenols and antioxidant capacity and also between content of ellagic acid and antiproliferative activity against bladder cancer T24 cells of humans. When the complex extract was compared with pure ellagic acid for antiproliferative activity, it was found that the complex extract has two times greater activity. This suggests that polyphenols work in synergies with the ellagic acids to counteract the proliferation of bladder cancer. Sandhya Rani Mandadi et al., The present research was aimed to formulate the herbal sanitizer, from Neem, Lemon juice and Tulasi. Phytochemical properties of neem extract, lemon juice, juice and Tulasi were analysed. Their zone of inhibition was checked against standard culture. Zone of inhibition of all the formulations was found. Formulated sanitizer was compared with sterillium sanitizer and it was found that sterillium was effective against E. coli organism. Hand sanitizers were effective against the test organism. The antimicrobial effectiveness was assessed by measuring the zone of inhibition against the test organism. Maximum inhibition (in mm) was seen in group A (Sterillium), i.e.,  $10.5 \pm 0.707$  and minimum in group B (Tulasi), i.e.,  $.0 \pm 0.707$ . The difference in the values of the different sanitizers was statistically significant. From the present study, it was concluded that sterillium was most effective and sanitizers prepared from herbal extracts was close and comparable.

Dr. k . P . Jai Ganesh et al., Current outbreak of Covid 19 has made us mandatory of using hand sanitizers everywhere. The hand hygiene is now being very essential in the public. Compliance with hand hygiene recommendations is critical to reducing colonization and infection of the hands of all people. Hand hygiene is of utmost importance because it may be contaminated easily from direct contact with airborne microorganism droplets from coughs and sneezes. Particularly in situations like pandemic outbreak, it is crucial to interrupt and cut the transmission chain of the virus by the practice

of proper hand sanitization. The use of hand sanitizers drastically reduce the transmission of microorganisms to patients, ultimately reducing morbidity, mortality, and costs associated with healthcare-associated infections (HCAI). It can be achieved with contact isolation and strict infection control tool like maintaining good hand hygiene in hospital settings and in public. The success of the hand sanitization solely depends on the use of effective hand disinfecting agents formulated in various types and forms such as bath soaps, water-based or alcohol-based hand sanitizer, with the latter being widely used in hospital settings. To date, most of the effective hand sanitizer products are alcohol-based formulations containing 70%–95% of alcohol as it can denature the proteins of microbes and the ability to inactivate viruses. The present study correlated with the data available and it will investigate the range of available hand sanitizers and their effectiveness as well as the formulation aspects, adverse effects, and recommendations to enhance the formulation efficiency and safety.

Hugo A.L. Filipe et al., Emergent diseases caused by viral and bacterial infections have proven to be a current and future challenge. The occurrence of these diseases is usually accompanied by the lack of vaccines and dedicated therapies leaving prevention as the best strategy to adopt. In that context, and apart from confinement and physical distancing measures, an increase in hygiene actions, namely hand and surface cleaning and disinfection can reduce the infection spread originated from our day-to-day routines. However, during crisis situations the high disinfectants demand can very likely lead to having them running out of stock. This impels many individuals and companies to produce their own disinfectants. Here, we explore the main components of a disinfection formulation, both for hand-rub and surface cleaning. Alcohol and non-alcohol based formulations are described, including the possibility to fine tune the properties of the final product in order to increase public acceptance while maintaining product efficacy. The action mechanisms of the main active principles are also described conjugating information from experimental and theoretical data. Overall, the main aspects to develop a disinfectant formulation are addressed, as well as their function, helping formulation developers to better understand the impact of their choices.

Ravindra B Malabadi et al., This literature review paper highlights the recent updates on the use of herbal extracts or essential oils of medicinal plants in the preparation of hand sanitizers. In India, Covid-19 patients with the development of black fungus infections, mucormycosis is another major health issue. Recent outbreak of coronavirus (SARS-CoV-2) with mucormycosis has promoted the hand hygiene so as to achieve a full recognition among healthcare workers, public and particularly elderly people for controlling the cross contamination of the pathogen. Hand hygiene can be achieved either through hand washing, or hand disinfection. Human health hazards are linked with the frequent use of alcohol-based hand sanitizers is a major health issue. The range of available 24 hand sanitizers and their effectiveness as well as the formulation aspects, adverse effects, and recommendations to enhance the formulation efficiency and safety. Adaptation of alternative preparations of hand sanitizers based on natural and plant resources are the possible solution to get rid off toxicity problem. Washing hands is one of the simplest, most effective ways to get rid of germs and avoid infection. Aromatic plants with essential oils have been used because of their many different biological properties, including antimicrobial properties. Therefore, herbal based hand sanitization has been promoted during the recent outbreak of SARS-CoV-2.

Jane Lee Jia Jing et al., Hand hygiene is of utmost importance as it may be contaminated easily from direct contact with airborne microorganism droplets from coughs and sneezes. Particularly in situations like pandemic outbreak, it is crucial to interrupt the transmission chain of the virus by the practice of proper hand sanitization. It can be achieved with contact isolation and strict infection control tool like maintaining good hand hygiene in hospital settings and in public. The success of the hand sanitization solely depends on the use of effective hand disinfecting agents formulated in various types and forms such as antimicrobial soaps, water-based or alcohol-based hand sanitizer, with the latter being widely used in hospital settings. To date, most of the effective hand sanitizer products are alcohol-based formulations containing 62%–95% of alcohol as it can denature the proteins of microbes and the ability to inactivate viruses. This systematic review correlated with the data available in Pubmed, and it will investigate the range of available hand sanitizers and their effectiveness as well as the formulation aspects, adverse effects, and recommendations to enhance the formulation efficiency and safety. Further, this article highlights the efficacy of alcohol-based hand sanitizer against the coronavirus.

PT Chavanke et al., 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 25 sanitizer has increased which causes less dryness to hand. 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, Glycerine etc. The estimated making cost of 100ml of sanitizer was 16 rupees. The mass production of this sanitizer can be very effective for large scale use of sanitizers by common people. Sayed, Ajaz Ahmed Kutub Ali et al., Hand sanitization is a crucial criteria in case of infection control. Hands are the prime culprit for the transmission of deadliest microorganisms to other body parts and other person as in communicable diseases. Application of Hand Sanitizers is significant in handling young infants, before preparing food, using bathroom, in hospitals (to impede nosocomial infections), etc. The present study used herbal drugs viz. Pomegranate peel, Lemon seed and Mace which comprises of active principle responsible for antibacterial activity. Extracted active principles were used to formulate an alcohol-based herbal hand sanitizer gel. The formulation was evaluated on the basis of various physical and microbiological parameters including homogeneity, pH and Viscosity testing; and In-vitro Antimicrobial activity by Agar plate diffusion method, Antimicrobial Susceptibility Testing and Determination of MIC respectively. The formulation was compared with the standard and gave promising results. This formulation might be useful in prevention of transmission of COVID-19 due to its dual merits, firstly its an alcohol based sanitizer and due to ethanol the envelop of the virus will be affected and hence killed of easily; secondly it contains Pomegranate peel extract which according to several studies as quoted by News -1 report is a known to degrade Corona virus 60 folds faster when given with Oseltamivir during the 26 emergence of Corona infection. Keywords: Hand sanitization; Herbal sanitizer; Antimicrobial activity; Pomegranate peel (*Punica granatum L.*); Lemon seed (*Citrus aurantiifolia* (Christm.) Swingle); Mace (*Myristica fragrans*); tannins; terpenes; COVID-19. Jerikias Marumure et al., The COVID-19 pandemic poses a severe threat to public health, resulting in high levels of mortality and morbidity. In response, there has been a significant usage of hand sanitizers in homes, public places, and healthcare systems. In the global panic, the market has a variety of products, and there are serious concerns about the safety and the potential of hand sanitization as the blue bullet for COVID-19. Therefore, this article presents a critical review of types of hand sanitizers available on the market, their active ingredients coupled with their mode of action in the wake of antiviral efficacies. In addition, the adoption of a culture of hand sanitization by society could raise the

demand for hand sanitizers for an extended period. The continuous use of hand sanitizers might pose some safety concerns. Consequently, the review articulates potential dangers associated with hand sanitizer used to equip suppliers and manufacturers with knowledge on the safety of different ingredients and formulations, hence safeguarding the final users.

Oyeniya, Y.J et al., The antimicrobial activity of Moringa olifera methanol leaf extract had been reported but with no effort to develop the extract into useful pharmaceutical products that is clinically relevant Objectives: This study aimed to develop an alcohol base herbal hand sanitizer containing synthesized Moringa silver nanoparticles as the active ingredient for use in personal hygiene and for combating the spread of outbreak of communicable diseases Methods: 10 mL of Moringa leaf extract (MLE), obtained by macerating the dried leaves with methanol was reacted with (40,30,20 and 10 mL) of 1mM silver nitrate solution to produced batches of Moringa silver nanoparticles (MSN). The morphology of MSN was obtained using a scanning electron microscope, while the particle sizes, 27 Polydispersity index and the zeta potential were obtained using a ZS-90 Zetasizer with dynamic and electrophoretic light scattering capabilities. MSN antimicrobial action was evaluated by agar diffusion method sequence to formulation of the herbal hand sanitizers, which were the evaluated for their effectiveness to reduce the microbial population. The color changes indicating the formation of MSN were within 10 minutes, all other MSN parameters significantly varied from batch to batch, ( $p \leq 0.05$ ) indicating the need of process optimization. The MSN were moderately dispersed, negatively charged and stable with PDI and ZP values ranging, 0.11-0.39 and 22-33 mv respectively. FA and FB with yields above 50 % and mean particle sizes of about 30 and 38 nm were selected for production scale up and formulation studies. The percentage microbial death for FA1 and FB1 were 100 % showing an improvement above the standard of 99.9 % microbial death. Conclusion: Alcohol base herbal hand sanitizers were successful formulated with synthesized MSN which demonstrated an improvement above the industrial standard with ability to eradicate microbial load by 100 %. These novel herbal hand sanitizers could be readily deployed to combat the spread of communicable disease outbreak like the current covid-19 pandemic.

Nandkishor.s et al., 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) from 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. The purpose of present study was to prepare herbal hand sanitizer incorporating the leaves extracts of Ocimum sanctum Linn.(Tulsi)and Eucalyptus globulus (Nilgiri), the well-known herbal combination with multidimensional activities; and to evaluate their respective antimicrobial efficacy and safety of hands. The formulation was evaluated against the specified 28 microorganism (Bacteria- E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis and Fungi- Saccharomyces cerevisiae, Candida albicans) by culture sensitivity test. The significance was found to be more in comparison to the standard reference. Miss. Snehal B Bhagat, et al., The main intention of this research is to prepare curcumin and Neem based herbal gel sanitizer by minimizing the alcoholic usage and to evaluate the anti-bacterial activity of this herbal sanitizer. Methods: curcumin and Neem gel sanitizer was prepared from the ethanolic extract of Curcuma longa and Azadirachta indica ( along with addition of gel base prepared from HPMC-E15. Results: The curcumin and Neem gel sanitizer was formulated and evaluated for its phytochemical constituents present in curcumin and neem, organoleptic properties, irritancy test and the efficiency of anti-bacterial activity of curcumin was also evaluated and it is safe and effective against pathogens. Conclusion: As a natural herb, curcumin and neem which was a household ingredient could also be effectively formulated as a sanitizer that reduces the side effects of alcoholic sanitizer products and is a best source that acts effectively against a numerous pathogens. Reni Mulyani, Lela L Khumaisah et al., During the Covid-19 pandemic, people are required to comply with health protocols, including frequently washing hands with running water or using hand sanitizers to minimize contamination of the Covid-19 virus. To increase the body's resistance or immunity against the Covid-19 virus, people are also required to consume nutritious food. In this community service, training will be conducted for Muhammadiyah and Aisyiyah residents of Sukabumi Regency so that they can make their own hand sanitizer that is environmentally friendly and inexpensive. Muhammadiyah and Aisyiyah 29 residents were also given knowledge about herbs or medicinal plants to increase body immunity against Covid-19.

---

## Materials & Methods

### 3.1 Collection of pomegranate peel

Fresh flowering twigs of pomegranate were collected from Kolli hills, Tamilnadu and authorised by The peel of the fruit is removed and it is shade dried for 1 week and it is made into fine powders for the extraction process.

### 3.2 Extraction of pomegranate peel

Fruits were collected from the local market and the peels were removed and washed thoroughly with distilled water. Peels were broken down manually into smaller parts and air dried. The dried peels were grounded with the help of grinder into fine powder. Accurately weigh 10g of the powder and transfer it into a 100 ml conical flask. 90 ml of ethanol was poured slowly to the flask and covered with Aluminium foil so as to avoid the loss of the solvent. The conical flask was shaken gently to mix all the contents for half an hour and kept in rotary shaker for 24 hrs at a speed of 80 rpm at room temperature. Following 24 hrs, the extract was filtered using Whatman No.1 filter paper. The filtrate was collected in a beaker. The contents of beaker (filtrate) was poured into a previously weighed petriplate and kept for evaporation in electric water bath. The dried extract was obtained in the petriplate. Petriplate along with the dried extract was weighed which was used to calculate the yield. The dried extract so obtained was stored in a beaker in refrigerator (4°C)

### 3.3 Extraction of lemon seed

The lemon seeds were removed and washed. Seeds were subjected to dryness at room temperature under darkness. The dried seeds were powdered using a grinder. 3g of this powder was weighed and transferred to a small conical flask. To this was added the solvent methanol 30ml. Gently shake the conical flask for 30 mins and keep it at 4°C for 24 hours. The extract was filtered using Whatman N°4 filter paper. The obtained filtrate was poured into a previously weighed petriplate and evaporated using electric water bath. Petriplate containing the dried extract was weighed and yield was calculated. Store the dried extract at 4°C.

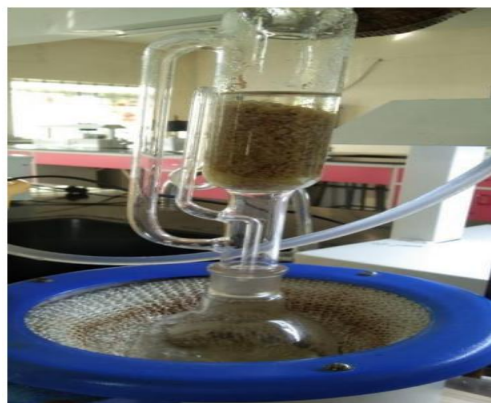


Figure 1 : Extraction of Lemon Seed

### 3.4 Extraction process by using soxhlet apparatus

Carbopol 940 is a carbomer which is an acrylic acid polymer. Carbopol 940 is a hydrophilic polymer. It acts as a gelling agent and bioadhesive. Carbopol 940 was mixed with distilled water with stirring. After the formation of homogenous mixture, the solubilizing agent triethanolamine (TEA) was added drop wise and stirred slowly with the help of glass rod to avoid formation of bubbles. While stirring it will be formed which was kept aside for 24 hrs. Extracts of Pomegranate peel and Lemon seed was added in Ethanol which was mixed with gel base. Followed by addition of glycerine. Tween 20 was added as a surfactant along with Methyl paraben as a preservative. Perfume was added in sufficient quantity to mask the smell of alcohol. Contents were stirred slowly and stored in air tight HDPE container.

Pharmacognostic evaluation reveals the presence of powdered characteristics that are very specific to the particular drug. It serves as a means of identification of the plant and also the part of the plant present. Pomegranate peel characters include Parenchymal cells which came from epicarp. Henceforth, it gives an indication that the part of the plant is peel. Lemon seed characters are endosperm, cotyledons, testa and tracheids. All these are the typical features of seed. Moreover, microscopic observation reveals that lemon plant is a dicot plant and presence of xylem vessels (tracheids) suggests that it's a vascular plant as well. The highest yield that was obtained was of Pomegranate peel with 52% yield in ethanol. Lemon seed in ethanolic extract gives 36% yield. Organoleptic Characteristics of all the formulations when formulated was from yellow to dark yellow colour, tinge of darkness adds on as the volume of the extracts added in the formulation increased. A drop or two of Lavendar gives the formulations a sweet fragrance. Homogeneity represents that the formulation is homogeneous. Viscosity of the formulation were found to be 50 centipascals. In-vitro Antimicrobial activity by Agar plate diffusion method against E. coli leads to the development of each of the sample added. Blank shows zone of inhibition of 15mm that is probably due to the antibacterial activity of alcohol added (ethanol). The formulation shows 20 mm zone of inhibition which was most effective. Antimicrobial susceptibility testing was done in our formulation. Formulated herbal hand sanitizer were found to be effective against E.coli. It is the major skin pathogen responsible for various ailments and illness. Hence, a concentrated formulation might be required to kill it.

Comparative study of herbal-based hand sanitizer and chemical-based hand sanitizer proves to have a comparable outcomes. Herbal-based hand sanitizer has a similar kind of effect as that of the chemical-based hand sanitizer. Hence, these can be used interchangeably. It's preferable to use herbal products as compare to chemical or synthetic due to various advantages. Stability studies showed that the formulation has the same organoleptic characters (colour and odour) and homogeneity from day 1 to day 15. However, the pH of the formulation has increased slightly but it is in still in range of skin's pH.

## Conclusion & Future Scope

The formulated herbal hand sanitizer using extracts of Pomegranate peel and Lemon seed showed strong antibacterial activity against the Gram negative bacteria E. coli. When compared with the chemical-based hand sanitizer, it was found that both of them were having comparable results and hence it can be concluded that the herbal formulation can act as a potential substitute for chemicalbased hand sanitizers. The latter having risk of side effects can be eliminated by making use of the formulated herbal hand sanitizer. Moreover, the natural components present in the formulated herbal hand sanitizer decreases the risk of side effects and if accidentally ingested by children, the components are digestible and will not cause any harm. A point of question over here is the presence of alcohol. Alcohol is having detrimental effects on ingestion. Therefore, researchers have made such a formulation which has the minimal amount of alcohol so that the bad effects of alcohol can be avoided. Also, as the alcohol concentration is low that

hands don't dry off too much. Punicalagin and terpenes are the major constituents helping fight off the tiny creatures and are not known to affect in anyway the human skin. Peel and seeds are often discarded as waste, and now it can be said that it's not a waste instead it's a "Warrior in disguise killing off the tiny rivals.

The formulated herbal hand sanitizer using Pomegranate peel and Lemon seed has shown drastic killing activity of the mentioned bacterial species. To know whether the formulation actives are broad spectrum, further studies can be conducted against various bacteria including both gram positive and gram negative. The formulation must be tested for activity against commonly infecting fungi and viruses. Finding minimum amount of sanitizer required to kill different microbes along with the time required can be done and used to categorize the microorganisms according to the susceptibility towards the sanitizer. So, one can identify whether the formulation is more effective against bacteria or fungi or virus. Comparison of the formulated herbal-based hand sanitizer with chemical-based hand sanitizer. Another future perspective can be simulating animal study to know the seriousness of accidental consumption of hand sanitizer by children. Thereby, evaluating the toxic effects in vivo. Finding solutions/antidotes is another area of research. An attempt can be made to formulate water-based or non alcohol-based herbal hand sanitizer and evaluating it on required parameters to characterize it can be done. Comparative study of antimicrobial activity of the formulated alcohol based herbal hand sanitizer and the newly formulated non alcohol based herbal hand sanitizer can be carried out to know the better one. Formulated herbal hand sanitizer can be subjected to Franz cell diffusion apparatus to analyse the penetration or permeation characteristics of the hand sanitizer through the skin of hands. An innovative approach would be analysing the activity of formulated herbal hand sanitizer against COVID-19. According to several studies as cited by News-1 website, punicalagin (a hydrolysable tannin from Pomegranate peel) is known to work hand-to-hand with Oseltamivir, an antiviral used to treat Influenza viral infections; and destroys Corona Virus 60% faster on the onset of Corona infection. This was explained by Chinese professor. Henceforth, our formulation has twin benefits of killing the novel Corona virus, that is firstly, ethanol works by affecting the viral capsid of the encapsulated viruses and Corona Virus is encapsulated as well. Secondly, the pomegranate peel extract contains punicalagin which can participate in killing the virus. The mechanism of killing COVID-19 by punicalagin can be researched. Further research can be carried out to confirm the killing activity of punicalagin alone against COVID-19. Study can be done to determine whether there is any change or not in the normal skin flora.

#### REFERENCES :

- [1] Shalini Malviya, Arvind, Alok Jha, et al. Antioxidant and antibacterial potential of pomegranate peel extracts. *AFSTI*.2014;51(12):4132-4137.
- [2] G. Karthikeyan and A.K. Vidya. Phytochemical analysis, antioxidant and antibacterial activity of Pomegranate peel. *RJLBPCS*.2019;5(1):218-231.
- [3] Hany M. Yehia, Manal F. Elkhadragey and Ahmed E. Abdel Moneim. Antimicrobial activity of pomegranate rind peel extracts. *African journal of microbiology research*.2011;4(22):3664-36668.
- [4] Arshad Husain Rahmani, Mohamed Ali Alsahli, Saleh Abdulrahman Almatroodi. Active constituents of Pomegranates (*Punica granatum*) as Potential Candidates in the Management of health through Modulation of Biological Activities. *Pharmacogn J*.2017;9(5):689-695.
- [5] Priyanka Kesur, Mayur Gahlout, Poonam B. Chauhan, et al. Evaluation of Antimicrobial properties of Peels and Juice Extract of *Punica granatum* (POMEGRANATE). *IJRSL*.2016;3(5):11-20.
- [6] Alessandra Masci, Andrea Coccia, Eugenio Lendaro, et al. Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. *Food Chemistry* 202.2016;pg no:59-69.
- [7] Entessar H.A. Al-Mosawe and Iman. I. Al- Saadi. The Extraction and Purification Of Gallic acid from the Pomegranate Rind. *Al-Mustansiriyah J. Sci*.2012;23(6):53-60
- [8] Awatef M Hasan, Ali Ali Redha and Qaher Mandeel. Phytochemical Investigations of pomegranate (*Punica granatum*) Rind and Aril Extracts and their Antioxidant, Antidiabetic and Antibacterial Activity. *Nat Prod Chem Res*.2018;6(4):1-10 50
- [9] Radwan S. Farag, Mohamed S. Abdel-Latif, Sekina, S. Emam, et al. Phytochemical screening and polyphenol constituents of pomegranate peels and leave juices. *LRJASS*.2014;1(6):086-093.
- [10] Sheng Wu and Li Tian. Diverse Phytochemicals and Bioactivities in the Ancient Fruit and modern Functional Food Pomegranate (*Punica granatum*). *Molecules*.2017;pg no:1-17.
- [11] K. Subashini. Review of Phytochemical Screening for Pomegranate Peel Extract Using crude, Aqueous, Ethanol and Chloroform. *IJESC*.2016;6(4):3329- 3332.
- [12] Jang-Gi Choi, Ok-Hwa Kang, Young-Soeb Lee, et al. In Vitro and In Vivo Antibacterial activity of *Punica granatum* Peel Ethanol Extract against *Salmonella*. *Hindawi Publishing corporation Evidence-Based Complementary and Alternative Medicine*.2009;2011:1-8.
- [13] Jyotsana Sharma and Ashis Maity. Pomegranate Phytochemicals: Nutraceutical and therapeutic Values. *Fruit, Vegetable and Cereal Science and Biotechnology*.2010;4(2):56-76.
- [14] Sreeja Sreekumar, Hima Sithul, Parvathy Muraleedharan, et al. Pomegranate Fruit as a Rich source of Biologically Active Compounds. *Hindawi Publishing Corporation Biomed Research International*.2014;2014:1-12.
- [15] Chaturvedi Dev, Shrivastava Rishi Raj Suhane Nidhi. Basketful benefit of Citrus Limon. *Int. Res. J. Pharm*.2016;7(6):1-4.
- [16] S. Halima-Mansour and R. Allem. Evaluation of antimicrobial activity of Algeria Lemon(*Citrus limonL.*) peels and seed extrasho. *Der Pharma Chemica*.2016;8(12):127-134. 51
- [17] Shweta Singh, Sonia Morya, Pinki Sainf, et al. Studies on Antioxidant and Antibacterial activity of Nutmeg Seed and Its Mace. *Advances in Life Sciences*. Print ISSN.2016;5(8):2278-3849,3207-3210.
- [18] Pardeep Kaur, Sanjeev Kumar Kataria, Balbir Singh, et al. Pharmacognostic Investigation of *Punica granatum L.* Peel. *Int J. Pharm. Drug. Anal*.2018;6(2):116-121.
- [19] Soni H, Nayak G, Mishra K, et al. Pharmacognostic and Phytochemical Evaluation of Peel of *Punica granatum*.2010;2(2):56-58.
- [20] Renu Narendra Jaisinghani, Shweta Makhwana and Ankit Kanojia. Study on antibacterial and flavonoid content of ethanolic extract of *Punica granatum* (pomegranate) peel. *Microbiology research*.

- [21] HPTLC fingerprinting of ethanol extract and fruit rind powder of *P. granatum*. [https://www.google.com/url?sa=t&source=web&rct=j&url=http://shodhganga.inflibnet.ac.in/jspui/bitstream/10603/134731/14/14\\_chapter%25206.pdf&ved=2ahUKEwimu9H\\_kpPqAhUmzzgGHZdgBCIQFjABegQIAhAB&usq=AOvVaw2Gec3J4L-oLgCh750gPvSP](https://www.google.com/url?sa=t&source=web&rct=j&url=http://shodhganga.inflibnet.ac.in/jspui/bitstream/10603/134731/14/14_chapter%25206.pdf&ved=2ahUKEwimu9H_kpPqAhUmzzgGHZdgBCIQFjABegQIAhAB&usq=AOvVaw2Gec3J4L-oLgCh750gPvSP). [accessed 21 June 2020]
- [22] Wagner H., Bladt S. *Plant drug analysis: A Thin Layer Chromatography Atlas*. Second edition. London:Springer;2009.
- [23] Dixit A, Pandey P, Mahajan R, et al. Alcohol Based Hand Sanitizers: Assurance and apprehensions Revisited. *RJPBCS*.2014;5(1):558-563.
- [24] Shaloo, Shayna Shafi, Simran Singh, et al. Formulation and development of mint containing herbal hand sanitizer. *EJPMR*.2017;4(11):454-457.
- [25] Dipti Singla and Kamna Saini. Formulation of an Herbal Substitute for Chemical Sanitizer and its Evaluation for Antimicrobial Efficiency. *IJCTR*.2019;12(3):114-120.