"PREPARATION AND EVALUATION OF HERBAL FORMULATION – HAND SANITIZER"

Submitted in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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Academic Year : 2019-2020

ABSTRACT:

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 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.

CERTIFICATE

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This is to certify that the project entitled **Preparation and evaluation of herbal formulation – hand sanitizer** is a bonafide work of **Quadri Shagufta Khatoon Mohammed Qamar Majeda Khatoon (Roll No.:16PH37) and Shaikh Mohammed Taqi Mohammed Ismail Shaheda Begum (Roll No.:17DPH66)** submitted for the appreciation of the degree of Bachelor of Pharmacy in Department of Pharmacognosy.

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Approval for Bachelor of Pharmacy

This project entitled Preparation and evaluation of herbal formulation - hand sanitizer by Quadri Shagufta Khatoon Mohammed Qamar Majeda Khatoon and Shaikh Mohammed Taqi Mohammed Ismail Shaheda Begum is approved for the degree of Bachelor of Pharmacy in Department of Pharmacognosy.

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Declaration

I declare that this written submission represents my ideas in my own words and where others ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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KEYWORDS AND GLOSSARY

Agar: A polysaccharide solidifying agent used in nutrient media preparations and obtained from certain types red algae (*Rhodophyta*). Both the type of agar and its concentration can affect the growth and appearance of cultured explants.

Agar well diffusion method: Agar well diffusion method is an antimicrobial activity test in which a hole is created in the agar medium and the extract solution is added to it.

Alcohol: An organic molecule containing an -OH group

Antibiotic: A class of natural and synthetic compounds that inhibit the growth of or kill some micro-organisms.

Antimicrobial agent: Any chemical or biological agent that harms the growth of micro-organisms.

Antimicrobial activity: Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes.

Antimicrobial susceptibility testing: A laboratory test which determines how effective antibiotic therapy is against bacterial infections.

Aril: An aril, also called an arillus, is a specialized outgrowth from a seed that partly or completely covers the seed.

Aseptic area: Asepsis or sterile is the state of being free of contaminating organisms (bacteria, fungi, algae and all micro-organisms except viruses) but not necessarily free of internal symbionts. The area free from any contamination is aseptic area.

Authentication: Authentication is the process of recognizing a user's identity.

Bacterium (bacteria, plural): A prokaryotic, single celled organism.

Chemical test: In these types of tests, chemicals are used as a part of the test procedure.

Communicable diseases: A communicable disease is an illness due to a specific infectious (biological) agent or its toxic products capable of being directly or indirectly transmitted from man to man, from animal to man, from animal to animal, or from the environment (through air, water, food, etc.) to man.

Comparative study: Comparative study is the study to demonstrate ability to examine, compare and contrast subjects or ideas.

Condensed tannins: Condensed tannins (proanthocyanidins, polyflavonoid tannins, catechol-type tannins, pyrocatecollic type tannins, non-hydrolyzable tannins or flavolans) are polymers formed by the condensation of flavans. They do not contain sugar residues.

Cotyledon: A cotyledon, or seed leaf, is a leaf that is stored in a seed. When the seed sprouts, the cotyledons are the first leaves that the plant has.

COVID 19: a mild to severe respiratory illness that is caused by a coronavirus (*Severe acute respiratory syndrome coronavirus 2 of the genus Betacoronavirus)*, is transmitted chiefly by contact with infections material (such as respiratory droplets) or with objects or surfaces contaminated by the causative virus, and is characterized especially by fever, cough, and shortness of breath and may progress to pneumonia and respiratory failure.

Dicot: Seed having two embryonic leaves or cotyledons.

Disease: The opposite of ease. Any alteration from the state of metabolism necessary for the normal development and functioning of an organism, usually associated with infection by a pathogen or the malfunction or absence of one or more genes.

Drug: A drug is a medicine or other substance which has a physiological effect when introduced into the body; a substance used in the diagnosis, treatment, or prevention of a disease or as a component of a medication.

Emollient: Emollients are moisturizing treatments applied directly to the skin to soothe and hydrate it. They cover the skin with a protective film to trap in moisture.

Encapsulated virus: A virus that has an outer wrapping or envelope. This envelope comes from the infected cell, or host, in a process called "budding off". During the budding process, newly formed virus particles become "enveloped" or wrapped in an outer coat that is made from a small piece of the cell's plasma membrane.

Endosperm: The part of a seed which acts as a food store for the developing plant embryo, usually containing starch with protein and other nutrients.

Epicarp: The outermost layer of a pericarp, as the rind or peel of certain fruits.

Escherichia coli (E. coli): A type of bacteria that commonly live in the intestines of people and animals. There are many types of *Escherichia coli*. Most pose no harm to human health, but one group produces a potent toxin which can cause food poisoning.

Evaluation: A systematic and objective measurement of the results achieved by a project, a program or a policy, in order to assess its relevance, its coherence, the efficiency of its implementation, its effectiveness and its impact, as well as the sustainability.

Extract: Extracts can be defined as preparations of crude drugs which contain all the constituents which are soluble in the solvent.

Extraction: Extraction is the method of removing active constituents from a solid or liquid by means of liquid solvent.

Formulation: A formulation is a mixture that has been designed as a useful product. Many products are complex mixtures in which each chemical has a particular purpose.

Fungus (fungi, plural): A eukaryotic, non-photosynthetic, spore-forming organism. They range from single celled organisms to very complex multicellular organisms.

Gel: Gels are semisolid systems consisting of dispersions of small or large molecules in an aqueous liquid vehicle rendered jelly like by the addition of a gelling agent.

Gelling agent: These are substances which, when added to an aqueous mixture, increase its viscosity without substantially modifying its other properties, such as taste. They provide body, increase stability, and improve suspension of added ingredients.

Hand hygiene / hand washing: Hand washing or hand hygiene is the act of cleaning one's hands with or without the use of water or another liquid, or with the use of soap for the purpose of removing soil, dirt, and/or micro-organisms.

Hand sanitizer: 1. A hand sanitizer, according to the latest FDA definition, is a supplement or alternative to hand washing with soap and water.

2. Hand sanitizer, also called hand antiseptic, handrub, or hand rub, agent applied to the hands for the purpose of removing common pathogens (disease-causing organisms). Hand sanitizers typically come in foam, gel or liquid form.

Hand washing: Hand washing is the act of cleansing the hands with water and soap for the purpose of removing soil or micro-organism (germs), in order to prevent cross-contamination and minimize nosocomial infections.

Herbal drugs: The term "herbal drugs" denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage.

Homogeneous: Homogeneous refers to the uniformity of the structure of matter.

Hydrolyzable tannin: A Hydrolyzable tannin or pyrogallol-type tannin is a type of tannin that, on heating with hydrochloric or sulfuric acids, yields gallic or ellagic acids.

Incubation: 1. The hatching of eggs by means of heat, either natural or artificial.

2. Period between infection and appearance of symptoms induced by parasitic organisms.

3. The culture of cells and organisms.

Incubator: An apparatus in which environmental conditions (light, photoperiod, temperature, humidity, etc.) are fully controlled, and used for hatching eggs, multiplying micro-organisms, culturing plants, etc.

Infection: The invasion of any living organisms by disease-causing micro-organisms, which proceed to establish themselves, multiply, and produce various clinical signs in their host.

Inoculate: Deliberately introduce something into. The process is inoculation. Not the same as *contamination.*

1. In bacteriology, tissue culture, etc., placing inoculum into (or onto) medium to initiate a culture.
2. In immunology, to immunize.

in vitro: Living in test tubes, outside the organism or in an artificial environment, typically in glass vessels in which cultured cells, tissues, organs or whole plants may reside.

Lemon: Lemon are obtained from *Citrus aurantiifolia (Christm.) Swingle* belonging to the family Rutaceae.

Mace: Mace are the arils of Nutmeg. Mace are obtained from *Myristica fragrans* belonging to the family Myristicaceae.

MIC: Minimum Inhibitory Concentration is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation.

Micro-organisms (microbe): A small living thing. The group includes bacteria, archaea, protozoa, algae, fungi and viruses.

Mobile phase: Mobile phase flows through the stationary phase and carries the components of the mixture with it.

Non homogeneous: Non homogeneous refers to the non-uniformity of the structure of matter.

Nonencapsulated virus: Cell parasites that do not have viral envelopes covering their central capsid.

Normal microbial flora: Microbes that have adapted to living on the body, are usually present and rarely cause harm.

Nosocomial: This term broadly covers any disease contracted by a patient while under medical care, but has come to be used synonymously with 'hospital-acquired' when referring to infections such as *Clostridium difficile,* Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa.*

Oil cell: Oil cells are plant cell organelles that serve as storage structures for lipids, primarily triacylglycerols.

Organoleptic characteristics: Organoleptic refers to evaluation by means of the organs of sense and includes the macroscopic appearance of the drug, its odour and taste, occasionally the sound or snap of its fracture and the feel of the drug to touch.

Pathogen: An organism that causes disease.

Peel: The skin or rind of certain fruits and vegetables.

pH: pH is a measure of hydrogen ion concentration, a measure of the acidity or alkalinity of a solution.

pH meter: It is an electric device used to measure hydrogen-ion activity (acidity or alkalinity) in solution.

Pharmacognostic evaluation /powder characteristics: It is a more detailed examination of a drug and it can be used to identify organized drugs by their known histological characters.

Pharmacognosy: Pharmacognosy is broadly defined as the scientific and systematic study of the structural, physical, chemical and sensory characters of crude drugs of vegetable, animal and mineral origin along with their history, method of cultivation, collection and preparation for the market.

Phytoconstituents/phytochemicals/chemical constituent: Medicinal plants contain chemical constituents that are used for their pharmacological properties.

Pomegranate: Pomegranates are obtained from *Punica granatum* L. belonging to the family Punicaceae.

Resistance: Term commonly used to describe the ability of an organism to withstand a stress, a force or an effect of a disease, or its agent or a toxic substance.

R_f value: Retention Factor is defined as the ratio of distance traveled by solute to distance traveled by solvent.

Seed: A seed is an embryonic plant enclosed in a protective outer covering.

Soap: 1. Soaps are water soluble sodium or potassium salts of fatty acids. Soaps are made from fats and oils, or their fatty acids, by treating them chemically with a strong alkali.

2. Soap is a cleansing agent created by the chemical reaction of a fatty acid with an allaki metal hydroxide. Soap has general chemical formula "RCOOX".

Solubilizing agent: Added to improve the solubility of poorly soluble drugs.

Solvent: A substance that dissolves a solute to create a solution.

Spectrum of activity: A product's range of antimicrobial activity.

Stability studies: Stability studies ensures the maintenance of product quality, safety and efficacy throughout the shelf life are considered as pre-requisite for the acceptance and approval of any pharmaceutical product.

Staphylococcus aureus (S. aureus): Bacterial species that is the cause of many different health problems in humans, including carbuncles, food-poisoning, and infections around medical devices and wounds.

Sterilization: A process of making a product sterile.

Stone cell: Brachysclereids or stone cells are short roughly isodiametric sclereids, occurring in the cortex, pith and phloem of stems (Cinnamomum), pulp of fruits (Pyrus).

Surfactant: Surfactants are substances that absorb to surfaces or interfaces, causing a marked decrease in surface tension.

Susceptible: The characteristic of a host organism such that it is incapable of suppressing or retarding an injurious pathogen or other factor.

Testa: The protective outer covering of a seed.

TLC: Thin Layer Chromatography can be defined as a method of separation or identification of a mixture of components into individual components by using finely divided adsorbent solid / (liquid) spread over a glass plate and liquid as a mobile phase.

Tracheids: Tracheids are part of xylem. These are elongated narrow tube-like dead and empty cells with hard thick and lignified walls with large cell cavity.

Transmission: Passing of a pathogen causing communicable disease from an infected host individual or group to a particular individual or group, regardless of whether the outer individual was previously infected.

Viral envelope: A spikey coat that covers the virus's protein coat or capsid.

Virus: An infectious particle that relies on the cellular machinery of the host cell to grow and replicate.

Viscosity: Viscosity is a quantitative measure of a fluid's resistance to flow.

WHO: The World Health Organization is a specialized agency of the United Nations responsible for international public health.

Yield: Amount of product obtained.

Zone of inhibition: This is an area of media where bacteria are unable to grow, due to presence of drug that impedes their growth.

1. INTRODUCTION

Hands are the prime source by which the microorganisms get access to our bodily systems. But to the local people the so-called "access" seemingly looks unimportant just because they don't understand the severity of the situation, they don't have medical knowledge and sometimes no facilities are available for maintaining hand hygiene. Collectively, it can be said that the local people are unaware. This unawareness may lead to severe tragedy that may outbreak in an unexpected manner. Communicable diseases are the diseases that are spread from one person to another. For instance, if the food handler's hands are contaminated and he serves foods or drinks to his customers, he is not only serving the eatables but also the pathogens that were present in his hands [27]. Therefore hand hygiene is essential. Hand hygiene is an important parameter that must be looked out by the scientists, physicians and all medical practitioners as well as clinical pharmacist as it plays a very critical role in case of nosocomial infections and increased antimicrobial multidrug resistance. Microorganisms especially bacteria (both Gram positive and Gram negative) such as *Escherichia coli, Pseudomonas spp., and Staphylococcus aureus* are responsible for nosocomial infections among which *Pseudomonas aeruginosa* is found prevalent in case of hospitalized and immunosuppressed patients [24].

Hands simulate the residence of number of microorganisms. Depending upon the location of residence within the skin layers, microbes residing on hands are classified into two major types i.e. Resident flora and Transient flora. *Corynebacterium diphtheriae , Staphyllococcus aureus, Staphylococcus epidermidis and Streptococcus viridans* are Resident flora that are present in the deeper layers of the skin, colonizing it. Resident flora possess low pathogenic potential and more resistance towards mechanical removal as well. On the contrary, *Staphylococcus aureus,* Gram negative bacilli and *Candida* species are Transient flora that are colonizing the superficial layers of the skin for a short period of time. The existence of transient flora in hands is due to contaminated environment and contact with patient (communicable pathogens). Transient flora are removable by mechanical means (washing hands) and are responsible for spread of infections and antimicrobial resistance [24].

Hand washing is a precaution that must be made mandatory for all. Washing hands lowers the number of pathogenic microorganisms and removes dirt. As per WHO recommendation, everyone should practice washing hands in all the following concerns: Preparation of food, eating food, visiting a sick person, treating a wound or a cut, using toilet and replacing diapers, cleaning a child who has used the toiler, after blowing nose, coughing or sneezing, after touching an animals or animal waste, after handling pet food or pet treats and after touching garbage. The common practice of cleansing hands is by using soap and water. The idea of hand cleaning emerges right from the beginning of 19th century. A French pharmacist in 1822 illustrated that solutions containing chlorides of lime or soda is known to abolish the disgusting odours of human corpses and henceforth such solutions can be used as a means of disinfectants and antiseptics [27].

However, soap and water are sometimes not available and therefore a ready-to-use product came into picture-hand sanitizer [28]. Hand sanitizer can be used as a substitute for soap and water and available in various forms such as liquid, gel and foam. Sanitizers with 70% alcohol kills 99.9% according to several studies [27]. Hand rubbing is preferred over hand washing and hence use of hand sanitizers is increasing day-by-day. The growing market contains a large number of chemical-based hand sanitizers, which are known to effect the normal skin on continuous use.

And if by any means, these chemicals get access to the systemic circulation may precipitate severe adverse effects and eventually risk the life of the consumers. Nature has solution to every problem. Plants are the rich source of secondary metabolites such as alkaloids, flavonoids, volatile oils, glycosides, etc which have various activities having importance in science and medicine. Herbal- based hand sanitizers incorporates the use of herbal drugs having optimum activity. The researchers have taken a step to formulate and evaluate a herbal-based hand sanitizer using Pomegranate peel and lemon seed.

Children are the special population and affected mostly by the various infections and this not only affects the health of the child but also it has an economic and social impact. Transmission is a major concern when it comes to children infections as in schools they may transmit these infectious particles to the classmates, teaching staff and in homes family members and any local public. This forms the major cause of absenteeism from school. An all time favourite hand sanitizer may now proof helpful and children will love rubbing their hands though. Interestingly, hand sanitizers will help reducing transmission rate and absenteeism rate as well [23].

Mechanism of working of alcohol based hand sanitizers is that alcohol causes denaturation of the lipids and hence causes dehydration in bacteria. However, in general hand sanitizers denature the proteins of the microbes. It is proposed that hand sanitizers don't work when hands are visibly dirty. Alcohols are effective against lipophilic enveloped viruses. Ethanol affects the viral capsid but the nucleic acid of the virus. Henceforth, it is hypothesized that they will only be effective in killing enveloped viruses namely Influenza virus, Hepatitis B virus, and Herpes simplex viruses 1 and 2 and not the enteroviruses namely nonenveloped human noro viruses (HuNov) [23]. Novel Corona virus is also an enveloped virus hence the formulated hand sanitizer will work to vanish it off and avoid its fast transmission and help humankind to get out of this world hijack.

Alcohol based hand sanitizers kill viruses, bacteria and fungus within seconds that is about 15 seconds is just required after rubbing the sanitizer in hand. For killing viruses it apparently requires 30 secs and may vary from virus to virus. It is a matter of subject. They prevent the transmission of seasonal flu, H1N1, colds and other bacterial and viral diseases. WHO is promoting the use of hand sanitizers and claimed it to be more efficacious than a hand wash. "A systematic review of publications between 1992 and 2002 on the effectiveness of alcohol-based solutions for hand hygiene showed that alcohol-based hand rubs remove organisms more effectively, require less time, and irritate skin less often than hand washing with soap or antiseptic agents and water." Is a WHO guideline pertaining to hand hygiene [23].

Pomegranate peels are obtained from *Punica granatum* L. belonging to the family Punicaceae. Pomegranate peel contains secondary metabolites such as Flavonoids, ellagitannins, proanthocyanidin compounds, minerals such as calcium, magnesium, phosphorus, potassium and sodium, Tannins like Anthocyanins and polyphenolic compounds such as Punicalagin [8]. Punicalagin has antibacterial activity. Pomegranate is believed to cure or prevent a number of diseases such as Cancers mainly bladder, breast, endometrial, prostate and lung cancers [10]. Not only Pomegranate is fournished with antibacterial activity but also it exhibits antifungal, antiviral, Anthelmintic, vermifugal, tanicidal, antiparasitic and molluscidal activities [13].

Figure 1: Pomegranate peels

Lemon seeds are obtained from *Citrus aurantiifolia (Christm.) Swingle* belonging to the family Rutaceae. It is also called as Mexican lime. It contains 21% protein, 39% vegetable oil and 29% fiber (lignin). It is used as a nutritional supplement and as a germicide [39]. It also contains limonoids [40]. Limonoids exhibit antibacterial properties [41].

Figure 2: Lemon seeds

Figure 3: Mace

Mace are the arils of Nutmeg. Mace are obtained from *Myristica fragrans* belonging to the family Myristicaceae. Mace consist of 7 to 14% essential oil and 30% fixed oil. Main chemical constituents of Mace are sabinene, α-pinene, myrcene, limonene, 1,8-cineole, terpinenol, myristicin, γ-terpinene, safrole, Malabaricone B and malabaricone C [38]. Antibacterial activity is due to Myristicin.

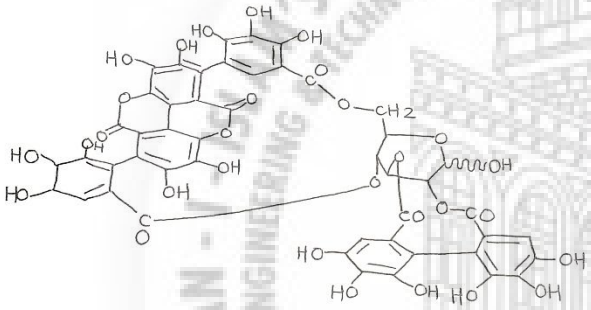


Figure 4: Punicalagin

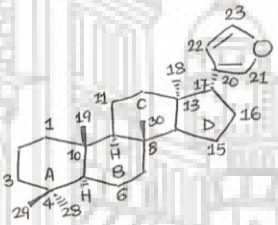


Figure 5: Limonoid basic skeleton

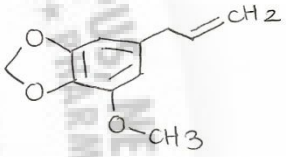


Figure 6: Myristicin

2. REVIEW OF LITERATURE

Shalini Malviya et al (December 2014) determined the yield, antioxidant potency, phenolic content and antibacterial potency with different solvents for extraction of Pomegranate peel. Among which highest yield was showed by ethanol : water (1:1). Highest antibacterial potency and phenolic content was found in 100% water and also in ethanol : water (7:3). Pomegranate peel extract shows maximum antibacterial activity against *Staphylococcus aureus* and nominal activity against *K. pneumoniae.*

G. Karthikeyan and A.K. Vidya (2019) 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.

Hany M. Yehia et al (16 October, 2011) studied the effect of combinations on antibacterial activity of pomegranate peel extract (PRE). When PRE was combined with ZnSO4, potentiated antimicrobial activity was found against *Bacillus subtilis*, *Staphylococcal* spp. and *Brucella* spp. PRE: Vitamin C (1:1) produced higher zone of inhibition againt *E. coli* and and *B. Indicus* among the 3 mixtures.

Arshad Husain Rahmani et al (2017) 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, 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 geranium* 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. megatarium* *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 (2016) extracted whole Pomegranate fruit blend. It was found that peel is rich in polyphenols, flavonoids, punicalaginq 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.

Entessar H.A. Al-Mosawe and Iman. I. Al- Saadi (2012) extracted gallic acid through Soxhlet extraction with the solvent system- ethanol : water (40:60). Phytochemical analysis (blue-black or green precipitate with ferric chloride reagent confirmed gallic acid), TLC (Rf value was found to be 0.9 with the mobile phase Toluene : ethyl acetate : formic acid in the ratio 3 : 3.5 : 0.5), HPLC (Retention time found was 3.496 min with mobile phase ethyl acetate : ethanol : water in the ratio of 1 : 5 : 4 v/v/v) and FT-IR (functions present were benzene ring, hydroxy groups and C=C double bond in Resveratrol structure) was done to characterize the extracted gallic acid.

Awatef M Hasan et al (July 09, 2018) did phytochemical screening on Pomegranate peel and arils and found to contain phenols, tannins, flavonoids, quinones, coumarin, steroids, triterpenoids and alkaloids. Pomegranate peel was devoid of Anthocyanins but present in arils. Flavonoids and phenolic content was higher in peels as compare to the rinds as resulted by quantitative assays. Polar extracts of peels and arils showed antioxidant activity. Aqueous extract of arils has significant antidiabetic activity. Methanolic and aqueous extracts of Pomegranate peel showed high antibacterial activity by disc diffusion method.

Radwan S. Farage et al (19 September 2014) did phytochemical screening and found its free-radical scavenging property. Polyphenolics were studied further qualitatively and quantitatively by HPLC analyzer.

Sheng Wu and Li Tian (21 September 2017) beautifully presented the use of Pomegranate in different ailments like Breast and Endometrial cancers, Prostate cancers, Colon and Bladder cancers, Cardiovascular diseases, obesity and aging. Phytochemical screening of the fruit was done and found to have Ellagitannins, gallotannins and derivatives, flavonoids, lignans, triterpenoids and phytosterols, fatty acids and lipids, organic acids and phenolic acids and compounds.

K. Subashini (April 2016) compared between the crude peel extract of Pomegranate and aqueous, ethanolic and chloroform extracts. Crude peel extract was found to contain more chemical compounds as compare to the other extracts.

Jang-Gi Choi et al (8 July 2009) performed in vitro and in vivo antibacterial activity of ethanolic extracts of Pomegranate peel against *Salmonella*. Various strains of *Salmonella* were used to determine antibacterial activity by disc diffusion method, MIC, Antibacterial Efficacy of PGPE in mice. Mice group infected with *Salmonella* spp and on consumption of Pomegranate peel survived and others died.

Jyotsana Sharma and Ashis Maity (29 October, 2010) prepared extracts and found Phytochemicals present in Pomegranate, showed its use in CVS diseases, hypertension, cancer, breast cancer, prostate cancer, lung and colon cancers. It has antioxidative properties. After immensely studying the whole plant of Pomegranate, it was found to have antibacterial, antifungal and antiviral activities. Also, found to have Anthelmintic, vermifugal, tanicidal, antiparasitic and molluscidal activities. Moreover, the sacred plant of Pomegranate proof to give protection against hepatotoxicity, Osteoarthritis, Alzheimer's disease. Pharmacokinetics study was done using animal models and in humans as well.

Sreeja Sreekumar et al (2014) presented a review article discussing about the chemical constituents of Pomegranate Fruit and tree, its therapeutic functions and its role as a nutraceutical.

Chaturvedi Dev et al (2016) studied *Citus Limon* plant as whole and did pharmacology study and find out it can be used in treatment of various ailments such as sore throat, prevention of kidney stones in kidney, supportive therapy for losing weight,etc. Lemon has anticancer activity, high content of Vitamin C and it can balance pH. Study does not revealed any adverse effects.

S. Halima-Mansour and R. Allem (2016) performed phytochemical testing of peels and seeds extract of Lemon and found to contain flavonoids which are the active principles responsible for antibacterial activity particularly inhibiting the growth of *S. aureus* and *E. coli*. They concluded that Lemon peels has profound antibacterial activity and can be used as an alternative to antibiotics.

Shweta Singh et al (2016) compares ethanolic and aqueous extract of Nutmeg and its Mace. Nutmeg ethanolic extract showed high TPC whereas Mace ethanolic extract showed low antioxidant activity. 50% ethanolic extracts of Nutmeg and Mace showed antibacterial activity in the following order *Shigella*>*S. aureus*>*Salmonella*>*E. coli*. 70% ethanolic extract inhibited the growth of *S. aureus* and *Salmonella*. 95% ethanolic extracts showed maximum activity against E. coli. 50% ethanolic extract of Mace was effective in killing E. coli, followed by *S. aureus*, *Shigella* and *Salmonella*. 70% ethanolic extracts of Mace gave maximum inhibitory action againts *Shigella*, then *Salmonella*, *S. aureus* and *E. coli*. 95% ethanolic extracts of Mace was effective in killing *S. aureus*. Camphor of Nutmeg and Myristicin of Mace was stated the active principles responsible for antibacterial activity by inactivating microbial adhesive enzyme and cell envelop protein. They concluded that ethanol extracts were far more effective in killing bacteria then the aqueous extracts.

Pardeep Kaur et al (2018) did the pharmacognostic evaluation of Pomegranate peel including morphological observations, microscopical observations, fluorescence analysis, determination of pH value and determination of flow properties.

Soni H et al (2010) performed microscopic studies, quantitative microscopy, total ash, water soluble and acid insoluble ash value of Pomegranate peel. Phytochemical tests proved that peel contains carbohydrates, tannins, glycosides and protein. TLC indicated the presence of punicalagin.

Shweta Makhwana et al conducted studies to determine flavonoid content and antibacterial activities of Pomegranate peel ethanolic extract. Pomegranate peel ethanolic extracts were found to have prominent antioxidant and antibacterial activities, and also good flavonoid content.

HPTLC fingerprinting of ethanol extract and fruit rind powder of *P. granatum*. The document contains information regarding TLC of Pomegranate peel, the detection reagents and the mobile phase used.

Wagner H., Bladt S. mentioned the detection reagents and mobile phase for performing TLC to confirm Myristicin. Reference R_f value stated was 0.75 to 0.8

Dixit A et al (2014) gave a brief introduction to hand sanitizers, advantages, disadvantages, working mechanisms, concerns related therein.

Shaloo et al (2017) formulated herbal hand sanitizer using Peppermint, Clove oil and Neem leaves extract and found to be effective in killing two bacterial species. It was not found to be effective against *Pseudomonas aeruginosa.*

Dipti Singla and Kamna Saini (2019) prepared herbal hand sanitizer using leaves of Eucalyptus, Sadabahar and Neem. Among this Eucalyptus aqueous extract with rose extract and glycerine was potent in killing germs.

Nandkishor S. Wani et al (2013) formulated herbal hand sanitizer using Tulsi leaves and Nilgiri leaves. It was effective in killing *E. coli*, *B. subtilis*, *S. aureus*, *C. albicans* but not effective for *P. aeruginosa* and *S. cerevisiae*.

Shri Balakrishna Acharya et al (2018) prepared water based herbal sanitizer using *Azadirachta indica*, *Ocimum sanctum* and *Citus limon*. It should effective killing of pathogenic germs of volunteers sample. It proved to have little or no side effects and hence safe.

Lusi Nurdianti et al (2015) formulated herbal hand sanitizer using *Aloe vera* with carbomer and HPMC base. Replica test showed that no of colonies made by formulation was more than the marketed preparation. Formulation with carbomer was more stable than HPMC base. Phenol coefficient for carbomer based formulation and HPMC based formulation was found to be 1 and 0.8 respectively.

Dr. R. Kalaivani et al (2018) made herbal hand sanitizer using *T. Copticum*, *A. calamus*, *P. nigrum*, *E. cardamomum*, *C. aromaticus* and *M. piperata*. Hand sanitizer had antimicrobial, antioxidant activity and considerable microbial load. It was more effective than alcohol bases hand sanitizers in terms of hygienic hands and softer hands.

Dian Riana Ningsih et al (2017) formulated hand sanitizer from soursop leaves. They prepared extract of soursop leaves using the solvent n-hexane. The extract was used to find MIC against P. acne and came out to be 1 ppm. Zone of inhibition reported is 0.7mm. Following this, they formulated 3 hand sanitizers of concentration 1,5 and 10 rpm which showed zone of inhibitions of 3.53, 3.26 and 2.20mm respectively.

X Fatima Grace et al (2015) formulated polyherbal hand sanitizer using Ginger, Lemon and Andrographis particulate and physical evaluation parameters were carried out like Colour, Odour and pH.

Rutuja Sunil Patankar and Dr. Nayna Chandak (2018) prepared different sanitizers using Lemon, Neem-Lemon and Neem. MIC, antibacterial activity was done and compared with sterillium and found to be more effective than that. Neem and Lemon juice extract also showed antibiofilm activity and hence can formulated as toothpaste or mouthwash liquid. Neem is bitter in taste hence cannot be used as hand sanitizer. The authors also did comparison of microbial load on hands before and after use of the formulated hand sanitizers and significant effect is marked.

Christina Osei-Asare et al (19 December 2019) prepared ethanol based hand sanitizer. Determined ethanol content in the final product and evaluated based on physical parameters. Microbial test against *Vibrio cholerae* shows that 63.70% hand sanitizer made from akpeteshie was found to be effective against the *Vibrio cholerae.*

Otokunefor, K; Princewill, I (Dec 2017) did in vitro evaluation of antibacterial activity of hand sanitizers. Three different sanitizers were tested against different microbes for antibacterial activity by Agar well diffusion test. All sanitizers were found to be efficacious since not the same for each bacterial strain.

Aryan Narang (October 2018) did a comparative study in which 5 different hand sanitizers were compared for their effectiveness against *E. coli*. 4 sanitizers were marketed formulations and one was home-made. Increasing order of strong activity as concluded by the author is Wellness Tree > Zuci Junior > Himalaya Pure Hands > Dettol > Home-made hand-sanitizer.

Zeeshan Afsar and Salma Khanam (2016) formulated herbal soap and hand sanitizer. Different extracts of *Cassia fistula*, *Milletia pinata* and *Ficus religiosa* were prepared using different solvents. Antimicrobial activity of these extracts showed that the ethyl acetate extract of the bark of *Cassia fistula* and *Ficus religiosa* and methanolic extracts of the bark of *Milletia pinnata* and *Cassia fistula* has significant activity. Henceforth, these extracts alone and in combination were used to formulate herbal soaps and hand sanitizers. Antimicrobial activity of the formulations showed that it has promising activity that can be understood by the Zone of Inhibition ranging from 18 to 26 mm. Various physicochemical test were done to characterize the formulations.

Mr. Amish J. Patel did the characterization of the plant *Echinops echinatus (*Roxb.*)*

Jinous Asgarpanah et al (June 2012) did characterization of Myristica fragrans Hoyutt.

E. Arriola et al (2006) determined different properties of Mexican lime *(Citrus aurantifolia swingle)* and listed its uses.

Onyilofe Sundy Enejoh et al (2015) identified the chemical constituents present in *Citrus aurantifolia (Chistm.) Swingle* plant as a whole and determined various nutritional and health benefits of it.

Aladekoyi G. et al (March 2016) did antibacterial activity determination of different Citrus seeds (Citrus Limon, Citrus Aurantifolia and Citurs Aurantium). All three exhibits strong antibacterial activity.

Seema Yuvraj Mendhekar et al (2017) did the characterization of *Myristica malabarica* Lamk.

3. AIM AND OBJECTIVE

- Waste production and climate change are the major environmental issues. Likewise accidental ingestion of chemicals (specially cleaning substances) by children; and microbial resistance are the prominent societal and medical issues emerging now-a-days. The present study was done with an aim to contribute solving the environmental, societal and medical issues by formulating and evaluating herbal based Hand Sanitizer.
- Rationale behind choosing herbal drugs are: ease of availability, relatively fewer incidence of side effects as compare to synthetic sanitizers, broad spectrum activity, these are economical and shares importance in perspective of users.
- The formulated herbal hand sanitizer comprises of Pomegranate peel, Lemon seed and Mace as the antibacterial agents. Pomegranate peels and lemon seeds are agro-waste which contribute to greenhouse-gas formulation resulting in climate change. Incorporating this so-called wastes in our formulation, now have a new avatar of benefiting the society.
- If formulation is accidentally ingested; Punicalagin, the major chemical constituent of pomegranate peel is a hydrolyzable tannin, meaning it is converted to ellagic acid in body and has strong antioxidant activity. Lemon seeds and Mace are safe as well. In contrast to chemical-based hand sanitizers, the major demerit of which is drug resistance; Punicalagin proofs to suppress methicillin resistance of *Staphylococcus aureus* to Oxacillin.
- With the increasing emergence of untreatable life threatening diseases such as AIDS, cancers and the novel Corona virus infection, there is an increasing demand for developing new therapeutic agents. 50% drugs prescribed by the physicians are derived from natural origin or synthesized by aids of natural models [37].
- World throughout is struggling with COVID-19, an attempt has been made to serve the society with our formulation which has dual merits as compare to the sanitizers already available in the market. According to the researchers, as cited by 'News 1' website- extract of Pomegranate peel is known to destroy Corona Virus 60 times faster when given with the antiviral agent Oseltamivir within 48 hours of emergence. Our hand sanitizer is alcohol based and hence knocks off the virus, moreover due to the presence of Pomegranate peel extract containing Punicalagin it may aid in destruction of virus- is our hypothesis and yet to be confirmed and hence it is a future scope.
- Objectives of the studies are:
    - Identification of the correct species of the plants chosen and authorizing them.
    - Pharmacognostic evaluation of the powders.
    - Extraction of the active principles in a suitable solvent.
    - Identification of the active/therapeutic constituents using TLC.
    - Formulation of alcohol-based herbal hand sanitizer.
    - Evaluation of the formulation
    - Comparative study with the chemical-based hand sanitizer
    - Stability studies

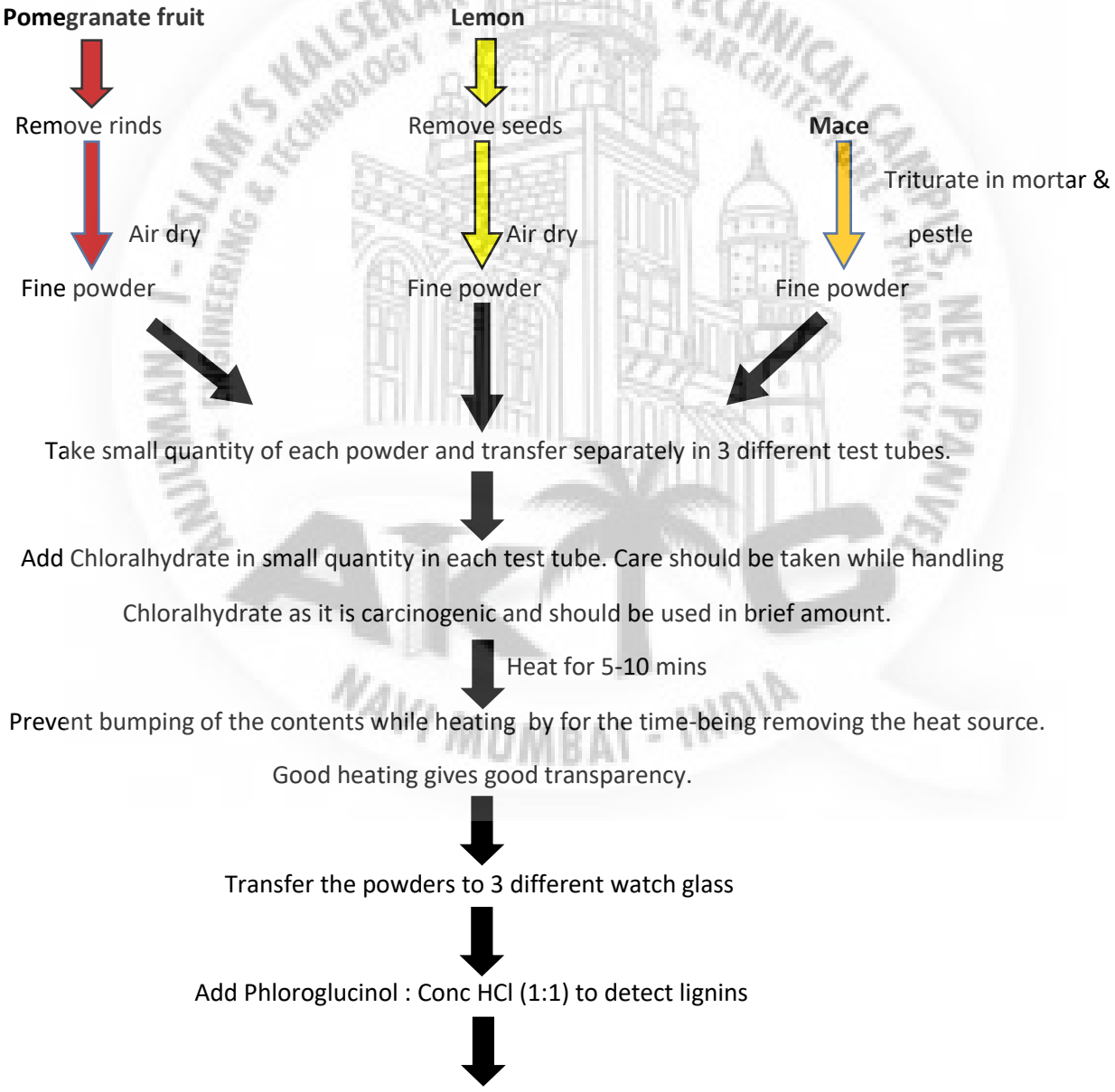
4. EXPERIMENTAL WORK

4.1 MATERIALS AND METHODS

4.1.1 Collection of samples:

Fresh flowering twigs of Pomegranate and Lemon were collected from New Panvel, Maharashtra, India and authorized by Blatter Herbarium, St. Xavier's College, Mumbai-1.

4.1.2 Pharmacognostic evaluation:



Spread powders in slides, add a drop of glycerine and cover with cover slip

↓

Observe under compound microscope at 10X magnification

↓

Search in different fields for the characters and identify them

4.1.3 Extract Preparation:

4.1.3.1 Pomegranate peel extract:

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) [20].

Figure 7: Pomegranate peel extract

4.1.3.2 Lemon seed extract:

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 [16].

Figure 8: Lemon seed

4.1.3.3 Mace extract:

Triturate 15 g Mace in motar and pestle. Transfer the powder in 50ml beaker. Add 30ml ethanol. Stir well. Wrap the opening of the beaker with foil paper. Keep it for 24 hours. Take petri plate, weigh it. It is initial weight of petri plate. Remove the foil paper. Filter the content using Whatman filter paper. Residue is discarded. Collect the filtrate in petri dish. Place the petri dish in electric water bath. Evaporate the solvent using electric water bath. Reweigh the petri plate. This is final weight of petri plate. Calculate the yield. Repeat the procedure using methanol and compare the yield.

Figure 9: Mace extract

4.1.4 Identification of phytoconstituents

4.1.4.1 Thin Layer Chromatography

4.1.4.1.1 TLC of Pomegranate peel

Prepare the mobile phase containing Toluene: Ethyl acetate: Formic acid (6:6:1). Keep it for saturation for half an hour. Meanwhile prepare the detection reagent. Detection reagent is Anisaldehyde-Sulphuric acid reagent. In 100ml beaker pipette out 0.5 ml of Anisaldehyde, followed by addition of 85ml Methanol. Subsequently add 5ml of Concentrated Sulphuric acid. Prepare the concentrated ethanolic extract of pomegranate peel and load the sample in the TLC plate. Put the TLC plate in the mobile phase and allow the solvent system to reach 80% of the TLC plate. Remove the plate, mark solvent front and air dry. Dip in Anisaldehyde- Sulphuric acid detection reagent. Heat at 100°C in hot air oven for 5-10 mins. Determine the R_f value and identify the spots [21].

4.1.4.1.2 TLC of Mace

Mobile phase was prepared in 100ml beaker by using 9.3ml Toluene and 0.7ml Ethyl acetate. Cover with petriplate. Keep it for saturation for 30 mins. Load the concentrated sample of ethanolic extract of Mace. Keep in mobile phase until the mobile phase reaches 80% of the TLC plate. Remove TLC plate. Mark the solvent front. Dip in freshly prepared 1% Vanillin in ethanol. Heat at 100°C for a minute. Remove and dip in 10% Sulphuric acid in ethanol. Heat at 100°C for 3-5 mins. Remove the TLC plate and find out the R_f value. It should be between 0.75-0.8 Observe under UV light and mark spots if required [22].

4.1.4.2 Chemical test for identification of hydrolysable tannin in Pomegranate peel extract:

To 1ml of the extract add 1ml 10% $FeCl_3$ aqueous solution. Hydrolysable tannins acquire blue colour and Condensed tannins acquire green colour. Punicalagin is the Hydrolysable tannin present in Pomegranate peel [8].

4.1.5 Formulation of Alcohol based herbal hand sanitizer gel

Figure 10: Gel base

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 dropwise and stirred slowly with the help of glass rod to avoid formation of bubbles. While stirring gel base will be formed which was kept aside for 24 hrs. Extracts of Pomegranate peel, Mace 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 containers [24].

Sr.No.	Ingredients	Quantity taken					Activity
		Formula No.1	Formula No.2	Formula No.3	Formula No.4	Formula No.5	
1.	Distilled water	9 ml	9 ml	9 ml	9 ml	9 ml	Vehicle
2.	Ethanol	2.5 ml	2.5 ml	2.5 ml	2.5 ml	2.5 ml	Antibacterial
3.	Pomegranate peel extract	0.1 ml	0.2 ml	0.3 ml	0.4 ml	0.5 ml	Antimicrobial
4.	Lemon seed extract	0.1 ml	0.2 ml	0.3 ml	0.4 ml	0.5ml	Antimicrobial
5.	Mace extract	0.1 ml	0.2 ml	0.3 ml	0.4 ml	0.5 ml	Antimicrobial
5.	Carbopol 940	0.15 g	0.15 g	0.15 g	0.15 g	0.1 5 g	Gelling agent
6.	Triethanolamine	0.62 ml	0.62 ml	0.62 ml	0.62 ml	0.62 ml	Solubilizing agent
7.	Glycerine	0.54 ml	0.54 ml	0.54 ml	0.54 ml	0.54ml	Emollient
8.	Tween 20	0.13 ml	0.13 ml	0.13 ml	0.13 ml	O.13 ml	Surfactant
9.	Methyl Paraben	0.15 g	0.15 g	0.15 g	0.15 g	0.15 g	Preservative
10.	Perfume	q.s.	q.s.	q.s.	q.s.	q.s.	Fragrance

Table 1: Formula for alcohol based herbal hand sanitizer gel

4.1.6 Evaluation

4.1.6.1 Physical Evaluation

The alcohol based herbal hand sanitizer gel was evaluated physically for parameters such as colour and odour.

4.1.6.1.1 Colour: Visual inspection.

4.1.6.1.2 Odour: Manual inspection [24].

4.1.6.2 Homogeneity test

All the formulations were spread in slides and observed under microscope. If the particles are dispersed uniformly, the formulation is said to be homogenous [28].

4.1.6.3 pH

Prepare 1% solution of the formulated herbal hand sanitizer using distilled water. Standardize the pH meter and determine the pH of the solution [24].

4.1.6.4 Viscosity Assay

To determine the viscosity, Brookfield viscometer is used. Place certain measured amount of the herbal hand sanitizer in a beaker. Immerse the tip of viscometer inside the sanitizer and determine the viscosity in triplicate [27].

4.1.6.5 In-vitro Antimicrobial activity by Agar plate diffusion method [24].

Figure 11: Aseptic working area

1. Prepare 100ml Nutrient Agar solution in conical flask by dissolving 2.8g of Nutrient Agar in Distilled water.
2. Put it in autoclave for 45mins. Also, keep 2 petriplates and borer in hot air oven for sterilization.
3. After 45 mins, remove Agar solution and cool it under running tap water.
4. After sufficient cooling pour it in petriplates provided all the operations being done in aseptic area by making use of two burners with a certain distance. Allow it to solidify.
5. Take two petriplates and label it.
6. Inoculate with E. coli . Allow to stand for 5-10 mins.
7. Make 4 wells in each petriplate.
8. 5 formulations, 1 blank (formulation without extracts) and 2 control (one in each petriplate) were filled one by one.
9. Incubate at 37°C for 24 hours. After 24 hours measure the Zone of Inhibition.

4.1.6.6 Antimicrobial Susceptibility Testing

This test is a function of the results obtained from Agar plate diffusion method. From the zone of inhibition data, identify the microorganism which is more susceptible to the killing activity of the herbal hand sanitizer. Likewise compare and find out the increasing order of susceptibility using zone of inhibition data of different microorganisms [29].

4.1.6.7 Determination of MIC

Minimum inhibitory concentration is defined as the minimum concentration required to completely diminish the growth of microbes. Inoculate the test bacteria (*E. coli*) in Petri plate of Mueller Hinton Agar. Bore it. Add varying concentrations of the herbal hand sanitizer. Incubate it at 37°C for 24 hours. The concentration showing clear zone of inhibition is referred to as MIC [27].

4.1.6.8 Comparative study of Herbal-based hand sanitizer and Chemical-based hand sanitizer

Hand samples were taken after application of both sanitizer using cotton swab and inoculated in solidified Nutrient Agar medium. Compare and contrast the results obtained. One having more no. Of colonies is less effective.

4.1.6.9 Stability studies

Herbal hand sanitizer was evaluated for organoleptic characteristics and homogeneity testing on 15 days of storage. The formulation found to have almost similar characteristics for 15 days was found to be stable [28].

5. RESULTS

5.1 EXPERIMENTAL

5.1.1 Pharmacognostic Evaluation

A. Pomegranate Peel Powdered Characteristics:
It showed the presence of many stone cells (astrosclerids) characterized by its pink colour. Also, oval to polygonal shaped Parenchymous cells of Epicarp were seen [18].

B. Lemon Seed Powdered Characteristics:
Endosperm was found scattered throughout along with cotyledons (dicot) and testa(seat coat). Tracheids (xylem vessels) were also found.

C. Mace Powdered Characteristics:
Oil globules were found dispersed in epithelial cells [42].

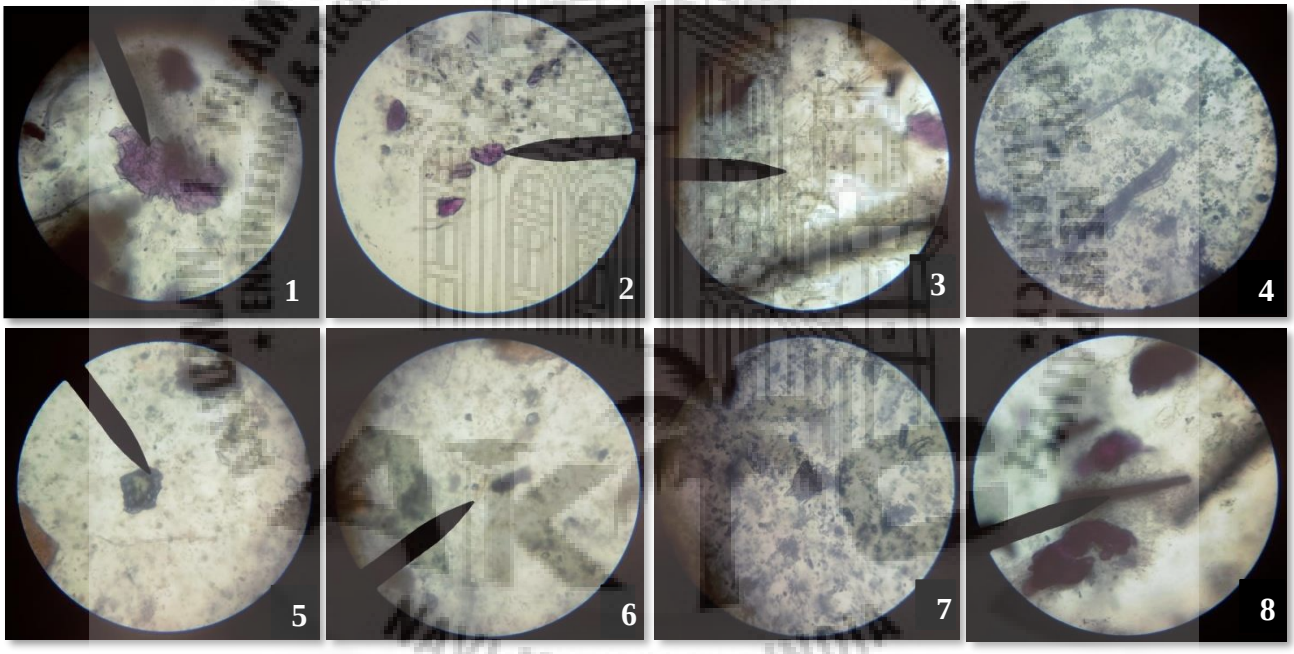


Figure 12: Powdered Characteristics 1,2- Group of stone cells of Pomegranate peel. 3- Parenchymous cells of Epicarp of Pomegranate peel. 4- Testa (seed coat) of Lemon seed. 5- Endosperm of Lemon seed. 6- Tracheids (xylem vessels). 7- Cotyledon (dicot) of Lemon seed. 8- Oil globules dispersed in Epithelial cells of Mace.

5.1.2 Yield Calculation

Drugs	**Solvents**	**Yield in grams** (Final wt of petriplate - initial wt of petriplate)	**%Yield** (Amount of dried extract /Amount of drug × 100%)
Pomegranate peel	Ethanol	5.2g	52%
Lemon seed	Methanol	1.0g	33%

Mace	Ethanol	5.4g	36%
Mace	Methanol	2.4g	16%

Table 2: Yield calculation

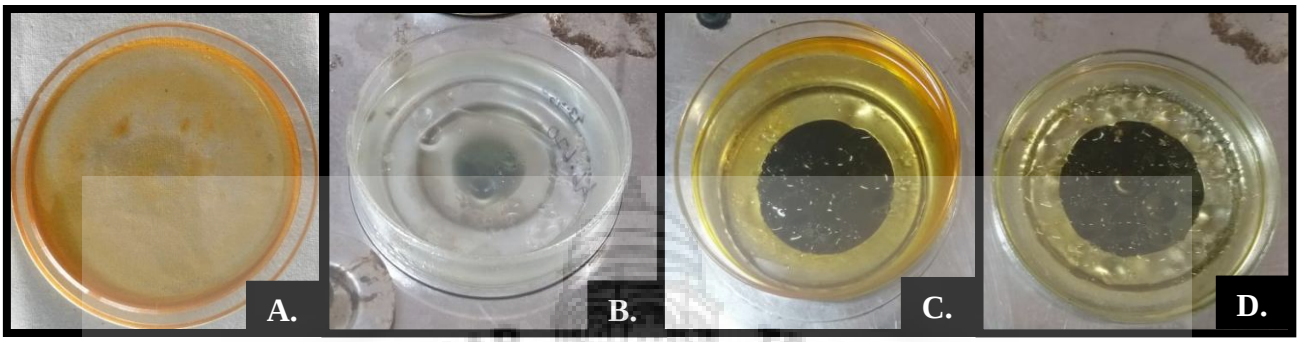


Figure 13: Yield A. Ethanolic extract of Pomegranate peel. B. Methanolic extract of Lemon seed. C. Ethanolic extract of Mace. D. Methanolic extract of Mace.

5.1.3 Physical Evaluation

Parameters	Observation
Colour	Bright yellow coloured formulation. Gradual increase in the depth of colour from Formula no.1 to 5
Odour	Characteristic odour of Vanilla

Table 3: Physical evaluation

Figure 14: Formulations

5.1.4 Homogeneity testing, pH and Viscosity Assays

Formulations	Microscopical observation	pH	Viscosity
Formula No. 1	Less dense. No Uniform dispersion seen	5.9	50 cPa
Formula No. 2	Different fields have non uniform distribution.	6	50 cPa
Formula No. 3	Spread throughout the slide. Most of the field view shows uniform dispersion.	6.1	50 cPa
Formula No. 4	Homogeneous	6.2	50 cPa
Formula No. 5	Highly homogeneous	6.2	50 cPa

Table 4: Homogeneity testing, pH and viscosity

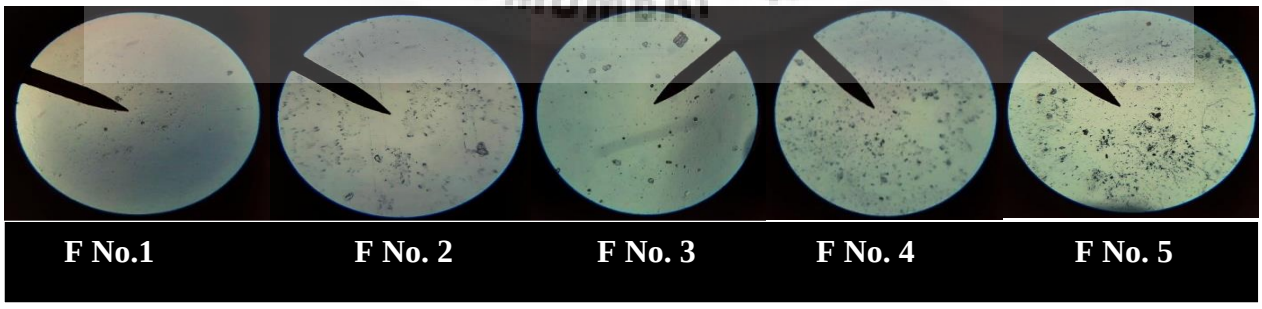


Figure 15: Homogeneity test

5.2 ANALYTICAL

5.2.1 Thin Layer Chromatography (TLC)

Sample	Mobile Phase	Detection Reagent(s)	Distance travelled by sample in cm	Distance travelled by solvent in cm	R_f value
Ethanolic extract of Pomegranate peel	Toluene:Ethyl acetate:Formic acid (6:6:1)	Anisaldehyde-sulphuric acid	1.5 cm 2.5 cm 3.0 cm 3.8 cm	5.5 cm	P1= 0.27 P2= 0.45 P3= 0.54 P4= 0.69
Ethanolic extract of Mace	Toluene:Ethyl acetate (9.3:0.7)	1% Vanillin in ethanol and 10% Sulphuric scid in ethanol	2.6 cm 3.1 cm 3.9 cm	5.5 cm	M1= 0.47 M2= 0.56 M3= 0.70

Table 5: Thin Layer Chromatography (TLC)

Figure 16: Pomegranate Peel

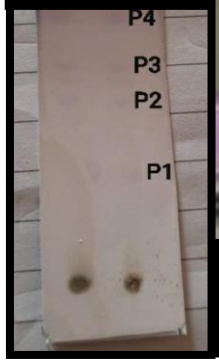
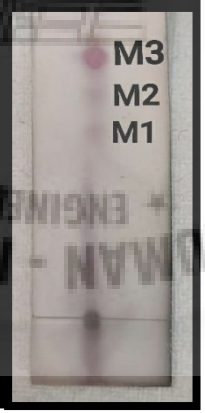


Figure 17: Running solvent system in TLC of Mace

Figure 18: Mace TLC



5.2.2 Chemical Test for identification of hydrolysable tannin in Pomegranate peel extract:

Blue colour confirms Hydrolysable tannins (Punicalagin) in Pomegranate peel.

Figure 19: Chemical test

5.3 MICROBIOLOGICAL

5.3.1 In-vitro Antimicrobial activity by Agar plate diffusion method:

Sr No.	**Sample** (Volume in mcl)	**Zone of Inhibition** (measured in nearest mm)
1	Blank	15mm
2	Formula No. 1	16mm
3	Formula No. 2	19mm
4	Formula No. 3	18mm
5	Formula No. 4	19.5mm
6	Formula No. 5	20mm
7	Positive Control I	21mm
8	Positive Control II	22mm

Table 6: In-vitro Antimicrobial activity by Agar plate diffusion

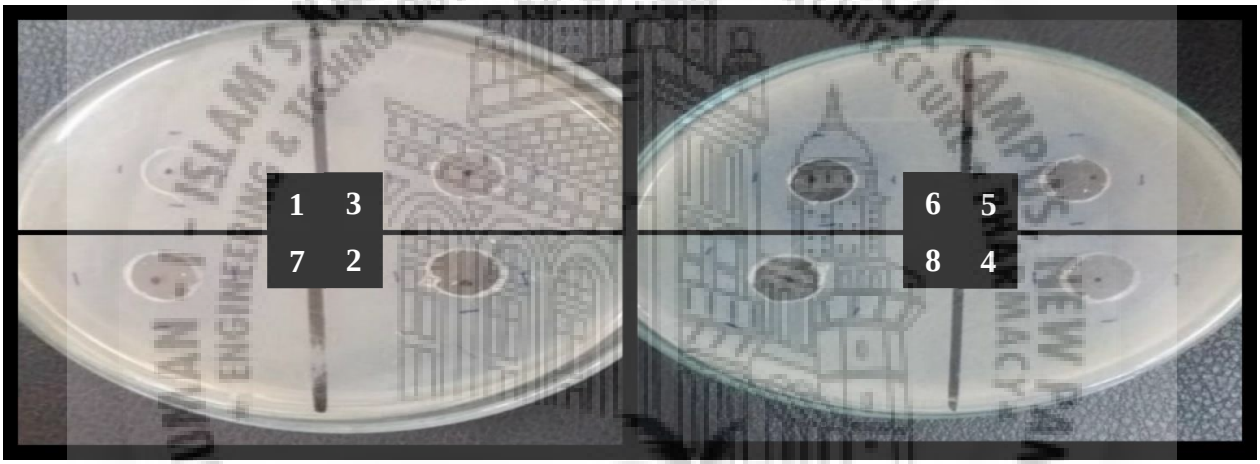


Figure 20: In-vitro Antimicrobial activity by Agar plate diffusion

5.3.2 Antimicrobial susceptibility testing

The formulated herbal hand sanitizer was found to be very effective against *E. coli*, has little activity against *S. aureus* and no activity against *P. aeruginosa*.

Microbial strain		**Zone of Inhibition (measured in nearest mm)**			
		Formula No. 2	**Formula No. 3**	**Formula No. 4**	**Formula No. 5**
E. coli	11mm	10.5mm	11.5mm		12mm
S. aureus	-	-	-		9mm
P. aeruginosa	-	-	-		-

Table 7: Antimicrobial susceptibility test

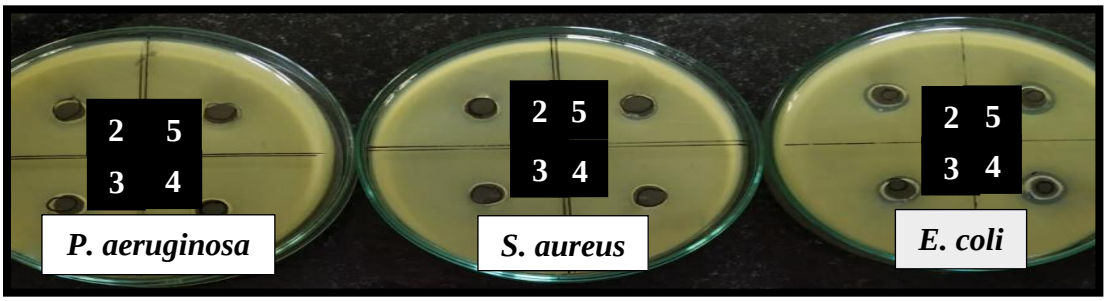


Figure 21: Antimicrobial susceptibility testing

5.3.3 Determination of MIC:

The concentration present in 0.2 ml of each extract (Pomegranate peel, Lemon seed and Mace) was found to be the minimum concentration at which the growth of *E. coli* was inhibited.

5.4 COMPARATIVE STUDY OF HERBAL-BASED HAND SANITIZER AND CHEMICAL-BASED HAND SANITIZER

Figure 22: Comparison of herbal-based hand sanitizer and chemical-based hand sanitizer

5.5 STABILITY STUDIES

Parameter	F No. 1		F No. 2		F No. 3		F No. 4		F No. 5	
	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
Colour	Y	D.Y	Y	D.Y	D.Y	D.Y	D.Y	D.Y	D.Y	D.Y
Odour	V	V	V	V	V	V	V	V	V	V
Homogeneity test	N.H	N.H	N.H	N.H	H	H	H	H	H	H
pH	5.9	6.1	6	6.3	6.1	6.2	6.2	6.4	6.2	6.4

Table 8: Stability study data Abbreviations: Y- Yellow, D.Y- Dark Yellow, V- Vanilla odour, H- Homogeneous, N.H- Non-homogeneous.

6. DISCUSSION

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 its a vascular plant as well. Mace characters are oil cells which can be correlated to its strong odour that comes essentially from the volatile oils located in these oil cells.

The highest yield that was obtained was of Pomegranate peel with 52% yield in ethanol. Lemon seed in methanolic extract gave yield of 33%. Constituents of Mace were extracted using two solvents viz. ethanol and methanol. Among the two ethanol outweighs methanol by 20%. Ethanolic extract showed 36% yield and methanolic extract showed only 16% 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 Vanilla gives the formulations a sweet fragrance. Homogeneity testing represents that Formula No. 1&2 are non-homogeneous while the rest were homogeneous. pH of all the formulations were found to be in between 5.9 – 6.2 that is in agreement with skin's pH (5.6).Viscosity of all the formulations were found to be 50 centiPascals.

As a method of analysis and identification of the phytoconstituents present in the prepared extracts of Pomegranate peel and Mace, TLC and biochemical test were used. TLC of Pomegranate peel illustrates the separation of 4 phytochemicals that were present in it's ethanolic extract that is marked in the figure with the abbreviations - P1, P2, P3 and P4 (Numbered ascendingly because the R_f value increase ascendingly). Identification of punicalagin cannot be done as the reference R_f value is unknown. On the contrary, TLC of ethanolic extract of Mace illustrates great separation of phytoconstituents. 3 spots can be clearly identified (M1, M2, M3) among which M3 having the R_f value of 0.70 is Myristicin as the Reference R_f value for the same is 0.75-0.8. 0.70 is close to this range and hence it can be said that the phytochemical separated was Myristicin. Chemical test confirms the presence of Hydrolyzable tannins in Pomegranate peel. The Hydrolyzable tannin present in Pomegranate peel is Punicalagin.

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). 16mm was measured for Formula No. 1. That means blank and Formula No.1 almost has the same activity and conversely, the extracts (0.1ml) were not much effective. It is more and less similar to the alcohol. Drastic increase in activity is seen in Formula No. 2 which has zone of inhibition of 19mm. Formula No.3 has zone of inhibition of 18mm which is having almost similar activity as that of Formula No. 2. Formula No. 4 and 5 were found to be the most effective formulations having zone of inhibition of 19.5mm and 20mm respectively.

Antimicrobial susceptibility testing was done only with 4 formulations as Formula No. 1 was having the same activity as that of blank. Formulated herbal hand sanitizers were found to be effective in the following order: *E. coli* > *S. aureus* > *P. aeruginosa*. Formula No. 2 to 5 were effective against *E.coli* in chronological sequence. But, only Formula No. 5 was effective against *S. aureus.* No formulations were effective against *P. aeruginosa.* This means that the *E. coli* is highly susceptible towards the antibacterial activity of the formulations, *S. aureus* is moderately susceptible towards the antibacterial activity of the formulations and *P. aeruginosa* is weakly or not at all susceptible towards the antibacterial activity of the formulations. *P. aeruginosa* is resistant to the effects of the formulated hand sanitizer. It is the major skin pathogen responsible for various ailments and illnesses. Hence, a more concentrated formulation might be required to kill it.

Minimum Inhibitory Concentration (MIC) was found to be the concentration of the extracts present in 0.2ml (Formula No. 2) against *E. coli*. Further analysis is required to find the exact the concentration.

Comparative study of herbal-based hand sanitizer (Formula No. 5)and chemical-based hand sanitizer proofs 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 all the formulations were having the same organoleptic characteristics (colour and odour) and homogeneity at Day 1 and Day 15. However, the pH of all the formulations had increased slightly but it is in still in range of skin's pH. The chart shows the comparison of pH at Day 1 and Day 15. All formulations are stable.

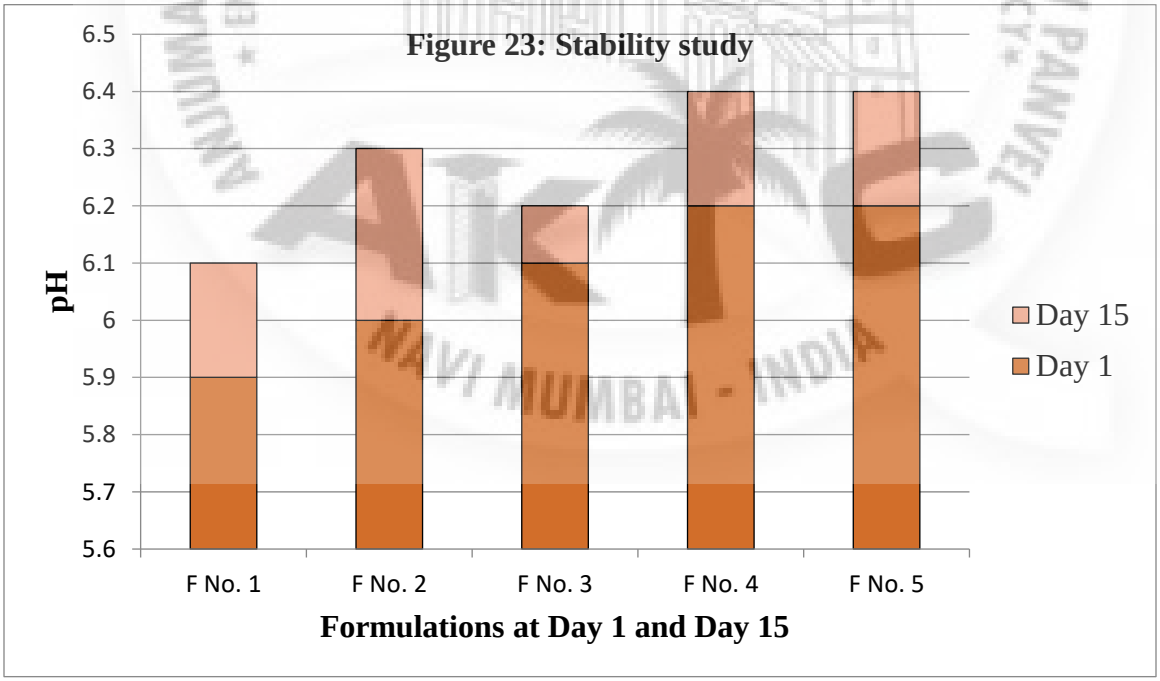


Figure 23: Stability study

7. CONCLUSION

The formulated herbal hand sanitizer using extracts of Pomegranate peel, Lemon seed and Mace showed strong antibacterial activity against the Gram negative bacteria *E. coli* and was also susceptible towards *S. aureus*. 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 chemical-based 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 accidently 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, terpenes and Myristicin 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, an now it can be said that it's not a waste instead it's a "Warrior in disguise" killing off the tiny rivals.

8. FUTURE SCOPE

The formulated herbal hand sanitizer using Pomegranate peel, Lemon seed and Mace 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.

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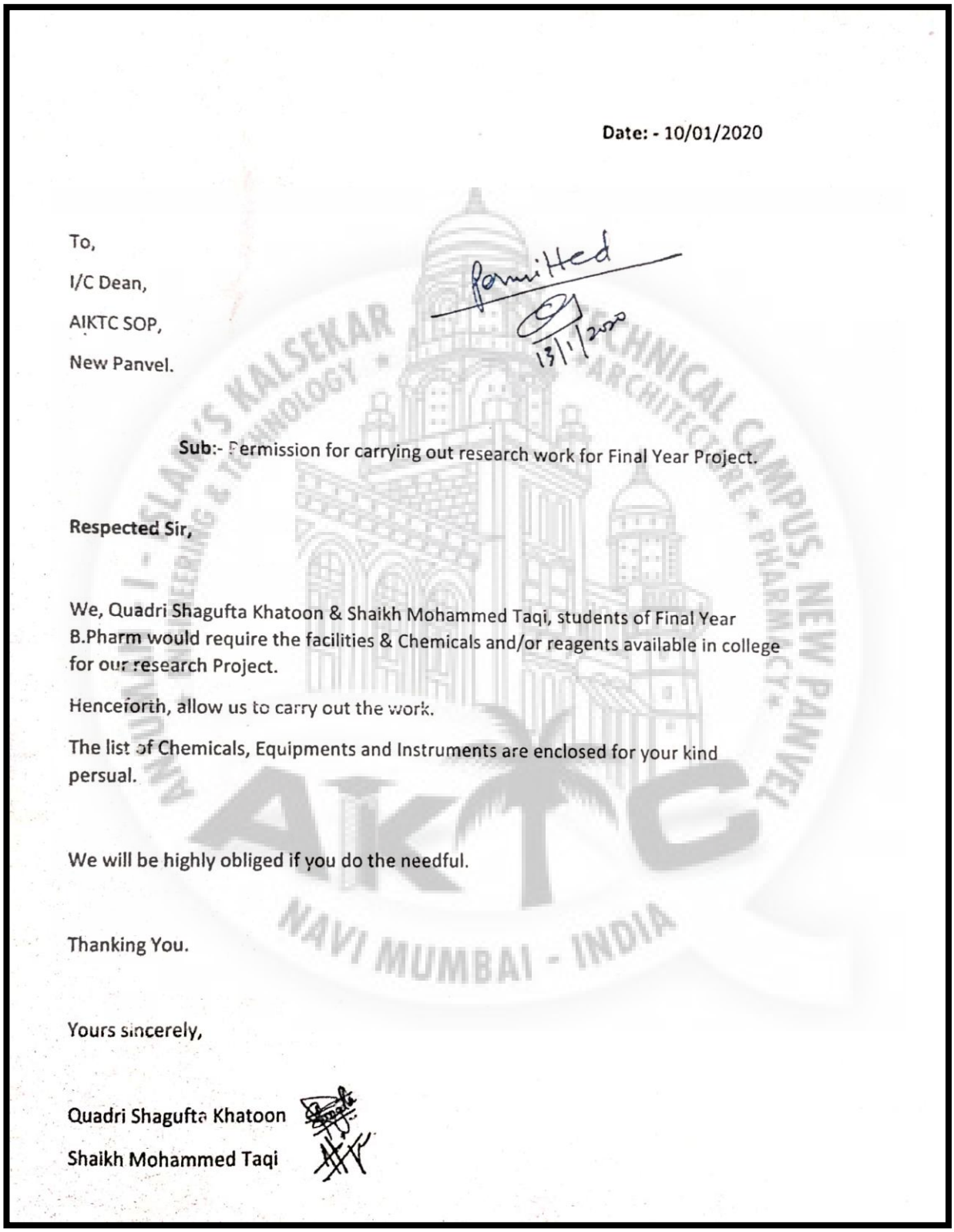
10. APPENDICES

Punica granatum and *Citrus aurantiifolia (Christm.) Swingle* were authorized by Dr. Rajendra D. Shinde, Principal, Head, Department of Botany & Director, Blatter Herbarium, St. Xavier's College, Mumbai-01 via Praveen sir. *Myristica fragrans* authentication request letter to Dr. Harshad M. Pandit. is attached herewith. Permission letter for conduction of research work was approved by Dr. Shariq Syed, Dean, SoP, AIKTC



Date: - 10/01/2020

To,

I/C Dean,

AIKTC SOP,

New Panvel.

Permitted

13/1/2020

Sub:- Permission for carrying out research work for Final Year Project.

Respected Sir,

We, Quadri Shagufta Khatoon & Shaikh Mohammed Taqi, students of Final Year B.Pharm would require the facilities & Chemicals and/or reagents available in college for our research Project.

Henceforth, allow us to carry out the work.

The list of Chemicals, Equipments and Instruments are enclosed for your kind persual.

We will be highly obliged if you do the needful.

Thanking You.

Yours sincerely,

Quadri Shagufta Khatoon

Shaikh Mohammed Taqi

Figure 27: Permission letter for carrying out research work

11. ACKNOWLEDGEMENT

I would like to take the opportunity to express my sincere thanks to my guide Ajaz Ahmad Sayed Kutub Ali, Assistant Professor, Department of Pharmacognosy, AIKTC, School of Pharmacy, Panvel for his invaluable support and guidance throughout my project research work. Without his kind guidance & support this was not possible.

I am grateful to him for his timely feedback which helped me track and schedule the process effectively. His time, ideas and encouragement that he gave is help me to complete my project efficiently.

I would also like to thank Dr. Abdul Razak Honnutagi, AIKTC, Panvel, for his encouragement and for providing an outstanding academic environment, also for providing the adequate facilities.

I am thankful to Dr. Shariq Syed , Dean, School of Pharmacy, Panvel and all my B.Pharm. teachers for providing advice and valuable guidance.

I also extend my sincere thanks to all the faculty members and the non-teaching staff and friends for their cooperation.

Last but not the least, I am thankful to all my family members whose constant support and encouragement in every aspect helped me to complete my project.

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