PREPARATION OF ALCOHOL BASED HERBAL HAND SANITIZERS AND ASSESSMENT OF ANTIMICROBIAL EFFICACY AGAINST SURFACE MICROORGANISMS

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Roll No.: 0119/05

Registration No.: 00655

Session: 2019-2020

A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Food Chemistry and Quality Assurance

Department of Applied chemistry and Chemical Technology

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Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

JUNE 2022

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This is to certify, that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made.

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JUNE 2022

DEDICATED TO MY HONORBLE TEACHERS, AND BELOVED FAMILY AND FRIENDS

PLAGIARISM VERIFICATION

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The author

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List of Abbreviations

$FeCl_3$	Ferric chloride
NaOH	Sodium hydroxide
H_2SO_4	Sulfuric Acid
$BaCl_2$	Barium Chloride
H_2O_2	Hydrogen per Oxide
IPA	Iso propyl alcohol
MIC	Minimum Inhibitory Concentration
MBC	Minimum bactericidal concentration
MHA	Muller Hinton Agar
BHI	Brain Heart Infusion
Ag NPs	Silver nanoparticle
ABHS	Alcohol Based Hand Sanitizer
TEA	Triethanolamine
rpm	Revolutions per minute

Abstract

The study is based on the formulation of new hand sanitizers that destroys germs as well as maintains skin health. For that reason, this study was focused on making alcohol based herbal hand sanitizers. The advantage of alcohol based herbal sanitizer is that it is free from side effects. The major objectives of this study were to formulate Twelve types hand sanitizers from nine types of plant extracts including Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata and two types of nanoparticles solutions using Isopropyl alcohol, glycerin, olive oil, honey, lemon essence, Hydrogen peroxide and distilled water as well as to assess the antimicrobial activity against three types of bacteria such as *E.coli*, *Salmonella* spp. and *Staphylococcus aureus* by comparing the sanitizers with blank hand sanitizer, Hexisol and market brand hand sanitizer. Moreover, there were other objectives including to identify nanoparticles forming plant by silver nitrate solution and plant extracts, to identify phytochemicals in plant extracts, to evaluate physical parameters such as color, odor, and pH of the formulated sanitizers. According to phytochemical tests, all the eight phytochemicals tested were found in Eucalyptus and Mahogany plant extracts but less than 8 phytochemicals were found in other plant extracts. The results of physical parameters have shown that the sanitizers exhibited standard pH and did not show any disagreeable color or odor. The antimicrobial tests revealed that the zone of inhibition against *E.coli* of Guava leaves hand sanitizer was the largest (31 ± 1·00 mm**)**; the zone of inhibition on *Salmonella* spp. of Eucalyptus nanoparticles sanitizer was greater (27·16 ± 1·04 mm**)** than any other hand sanitizers; Mix herbs hand sanitizer had the greatest zone of inhibition on *Staphylococcus aureus* (19·16 ± 1·04 mm**)**.The zones of inhibition of all sanitizers are larger than blank sanitizers. Indeed, it is proven without plant extract the zone of inhibition is small. In addition, zone of inhibition of Sepnil (market brand sanitizer) against *E.coli, salmonella* spp. and *Staphylococcus aureus* were 21·16 ± 1·25, 20·5 ± 0·5, 12·16 ± 0·76 mm respectively which are less than the previously mentioned herbal hand sanitizers. Again, the zone of inhibition of Hexisol against *E.coli, Salmonella* spp. *and Staphylococcus aureus* were 34·33 ± 1·52, 38·5 ± 1·32, 28·5 ± 0·5 mm respectively which were greater than previously mentioned herbal hand sanitizers. Though the zone of inhibition of Hexisol is greater against all three bacteria, it contains only chlorhexidine gluconate and Isopropyl alcohol which may cause dryness, irritation

and allergens. Sepnil also contains synthetic product which may be harmful to skin. Therefore, the formulated hand sanitizers are better than Hexisol and Sepnil in many purposes.

Keywords: Plant extracts, Alcohol based herbal hand sanitizers, Phytochemicals, antimicrobial activity, Zone of inhibition.

Chapter 1: Introduction

1.1 Background of the study

The definition of hygiene is the practice of keeping oneself clean, which is very important in maintaining one's health. A healthy lifestyle necessitates maintaining bodily hygiene and utilizing a cleaner. These ideas emphasize the need of maintaining good hygiene in illness prevention (Kevany and Huisingh, 2013). One of the most important measures for minimizing the risk of infectious agent transmission via contact and fecal-oral routes is skin cleanliness, particularly hand hygiene (Peng et al., 2020). Hands are the first point of contact for several germs and diseases. So, hand cleanliness is the most important step for preventing infections and preventing the spread of hazardous bacteria. Moreover, hand cleanliness is the single most effective, simple, and accessible system of avoiding nosocomial infections (Trampuz and Widmer, 2004). Infections acquired in a hospital or health-care environment are known as nosocomial infections. These infections are the outcome of a large pathogen epidemic, the development of weakened hosts, and efficient transmission pathways from patient to patient (Safdar et al., 2005). For that reason, the number of nosocomial infections is on the rise and has become a major problem in hospital treatment, resulting in longer hospitalization, widespread sickness and mortality, and exorbitant expenditures (Wilson, 2008.). Opportunistic bacteria such as Escherichia coli, and Staphylococcus aureus are frequently implicated in nosocomial infections. Surgical wounds, urinary tract infections, skin infections, respiratory tract infections, gastrointestinal tract infections, blood infections, and central nervous system infections are some of the most common infectious locations. These bacteria also tend to infiltrate health-care employees' natural flora. (Elliott and Justiz-Vaillant, 2018). Filthy hands can act as vectors for transmitting microorganisms. When food handlers defile their hands and subsequently distributes these bacteria to customers by hand contiguity with food or drinks, pathogenic organisms responsible for outbreaks are disseminated from the food handler's hands to others. Following the consumption of these germs, the customer is exposed, which may induce gastrointestinal disease. Pathogens infiltrate the food supply through hand contiguity with ready-to-eat foods, which is a particularly significant method. Food handlers who come into contact with unwrapped items that will be eaten raw or without further cooking or preparation have been classified as a high-risk category. (Shah et al., 2014). Currently, two fundamentally distinct hand-hygiene processes are in use: hand washing and alcohol hand-rub. Hand washing entails using an antimicrobial soap, non-antimicrobial or and creating mechanical friction by rubbing hands together for 1 minute, then rinsing with water and drying thoroughly with a disposable towel. Instead of water, alcohol is used in the alcohol hand-rub process. Unlike hand washing, the goal of alcohol hand-rub is to destroy all transitory and most resident flora, resulting in a more effective and quick decrease of skin flora. (Kolhapure, 2004). Hand sanitizers are elements that function as both cleansing and disinfection agents. Hand sanitizer is a liquid that is often used to keep living things clean (Golin et al., 2020). Sanitizers which is alcohol-based commonly comprise of some combination of iso propanol, ethanol or n- propanol. Most abuzz versions hold 60 to 95% alcohol (Fiorillo and Romano, 2020) Different research expressed that, sanitizers with at least 70% alcohol were suggested to kill 99.9% of the bacteria on hands (Ochwoto et al., 2017). Isopropyl alcohol kills 99.99 percent or more of all non-spore generating bacteria in less than 30 seconds in the lab or on human skin. After 30 seconds, alcohol rubs/sanitizers with a 70% alcohol concentration (ethyl alcohol) eradicate 99.99 percent of germs on hands, and 99.99 percent to 99.999 percent in one minute (Ichor et al ; 2018). In health care settings, hand sanitizers are preferable over soap and water. It kills germs more effectively and is more tolerable than soap and water (Arbogast et al; 2016). When skin is exposed to soap for an extended amount of time, the pH of the skin can rise to 7.0-8.5 and stay there for 3-4 hours. Soaps and detergents, particularly anionic or cationic versions, are the most harmful of all the compounds that are applied to the skin on a regular basis (Fluhr et al., 2008). Alcohol kills bacteria by damaging cell membranes and denaturing their proteins. Gram negative bacteria (such as *E.coli* and *Salmonella*) are more vulnerable to sanitizers because they have a weak peptidoglycan cell wall covered by an outer membrane that may be destroyed by alcohols, according to scientists. Gram-positive bacteria (*Staphylococcus aureus*) have a stronger peptidoglycan cell wall, making them more resistant to alcohol-based sanitizers. (Acharya et al., 2018). Plants were the primary treatment for a variety of ailments before to the development of modern medicine. Microbes gradually acquire resistance to various antibiotics as they become available. Researchers are drawn to plants with antibacterial capabilities as a result of these findings. They strive to take use of secondary metabolites' particular capacity to have long-lasting and sustained action against a wide range of microorganisms. (Hutchings et al., 2019). Plants have traditionally been employed by successive healers to prevent or treat infectious situations. Secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids are plentiful and diverse in plants. Antimicrobial activities have been discovered in vitro for several secondary metabolites (Chahal et al., 2021). Metallic nanoparticles are the most promising of the nanoparticles used for all of the above purposes because of their noticeable antibacterial feature caused by their massive surface area to volume ratio, which is of interest to researchers due to the growing microbial resistance to metal ions, antibiotics, and the improvement of resistant strains (Ahmed et al., 2016). Silver nanoparticles are an ancient nanotechnology product that has piqued interest caused by their unique properties such as chemical durability, excellent conductivity, catalytic, and, most importantly, antibacterial, antiviral, antifungal, and anti-inflammatory functions that can be incorporated into composite fibers, cryogenic superconducting materials, cosmetic products, food industry, and electron transport (Kumar et al., 2009).

1.2 Description of the problem

Human hands are the media which can easily transmit health hazardous organisms by touching the daily accessories. Even germs can get onto foods from hands during eating. The uses of hand sanitizer in higher education place, health care and in some urban area is available but in rural area the uses of hand sanitizer are rare. In places where soap and water are not available, people eat food without washing their hands and get infected with deadly diseases. For this reason, low-cost hand sanitizers are needed so that people can easily clean their hands at low cost. Furthermore, certain commercial sanitizers including Hexisol include synthetic disinfectants that can dry up and harm the skin. As a result, hand sanitizers created with natural components and skin-beneficial compounds will be superior to other hand sanitizers.

1.3 Significance of the Study

The significance of the study is to formulate Alcohol based herbal hand sanitizers which will kills germs as well as keep hands moist and soft. For that reason, olive oil, glycerin, and honey were used in this formulation along with alcohol and plant extracts. Generally, in market brand hand sanitizer synthetic derivatives have been used as moisturizing agents and disinfecting agents. But in most cases, these synthetic hand sanitizers irritate and dry the hands. Thus, using natural extract can greatly reduce the harmful effects of synthetic ingredients. The main benefit of using natural source is that they are easily available, inexpensive and mild compared to chemical products. In this study the herbal extract is used which is rich in secondary metabolites such as alkaloids, tannins, flavonoids, saponins, phenolic compound, carbohydrate, acidic compound. Again, these secondary metabolites help to reduce germs on hand and harmless to hand and alternative to synthetic drugs. Moreover, nanoparticle sanitizers are also used in this study because nanoparticle has antibacterial activities.

1.4. Objectives

1. To prepare plant extracts from 9 different herbs such as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava and Gulancho lata using methanol solution formulating hand sanitizers and identifying the presence of phytochemicals in the plant extract.

2. To identify nanoparticles forming plants using silver nitrate solution and plant extract.

3. To perform research and development process for suitable hand sanitizer according to solubility of the ingredients such as IPA, olive oil, honey, glycerin, H_2O_2, and lemon essence in different ratios.

4. To formulate 12 types of herbal hand sanitizers and evaluate physical parameters such as color, odor, pH of the hand sanitizers.

5. To compare the 12 types of hand sanitizer with blank hand sanitizer, Hexisol and market brand hand sanitizer to find the best one in accordance with antimicrobial activity against 3 types of bacteria such as *E.coli, salmonella spp.* and *staphylococcus aureus.*

Chapter 2: Review of Literature

2.1 Hand sanitizer

Hand sanitizer, often known as hand antiseptic, is a complement to or replacement for handwashing with soap and water (Dyer et al., 1998). Hand sanitizers are not a substitute for full hand washing. Instead, it is advantageous when hand cleaning is not possible (Vessey et al., 2007). It is thus a simple thing to eradicate and limit germs on hands by washing hands with water and adding particular components. Along with an increase in people's activities, particularly in metropolitan areas, and a plethora of fast items, hand sanitizers are an innovative waterless hand cleaning (Verma et al., 2013). To wash hands with soap and then rinsing them with water to remove the soap has long been the standard method of hand care. Antimicrobial products, on the other hand, do not need to be rinsed since they evaporate when they come into contact with water. As a result, these items are referred to as rinseless hand sanitizers (Golin et al., 2020). In recent years, they've gained popularity as a new technique to sanitize hands. When using a rinse less hand sanitizer, the user applies the solution to his or her hands and rubs them together, allowing the liquid to evaporate or absorb into the skin. Lotions, liquids, gels, and foams are all examples of antimicrobial products. Alcohols, trichlorohydroxy diphenyl ether (Triclosan), and parachloro metaxylenol (PCMX) are antimicrobial agents that may be utilized in such goods (Rotter, 1999). Hand sanitizers are obtainable in watery, foam, and easy-to-apply gel formulations. They are employed to the palm o and rubbed over whole hands and fingers until dry. Doctors, surgeons before and after surgery, pathologists, and researchers, as well as restaurants, toiletries, and other companies, commonly use the product. In the laboratories of medical and applied medical science institutes, hand sanitizer is also supplied, which students use after each practical session (Surwase et al., 2021). Alcohol has been used as an antiseptic since at least 1363, with proof of its effectiveness only being apparent in the late 1800s (Thorat et al., 2020). Traditionally, hand sanitizers were separated into two types: alcohol-based and non-alcohol-based. While 62 percent alcohol-based hand sanitizers were formerly common, most alcohol-based hand sanitizers today contain between 60 and 85 percent alcohol (Golin et al., 2020). Hand sanitizers with alcohol act against a comprehensive range of germs but not spores. To repose the skin from drying out, some versions include ingredients like glycerol (Jing et al., 2020). Although alcohol-based formulae that meet federal composition guidelines are typically regarded effective, alcohol-based antiseptic hand wash preparations are combustible and have no antibacterial activity that lasts. Additionally, frequent use might cause skin dryness and irritation (Dyer et al., 1998). Benzalkonium chloride, non-alcohol based hand sanitizers, are known to have minimal effectiveness against gram negative bacteria when compared to alcohol and is susceptible to pollution by previously mentioned germs (Ali et al., 2015).

2.2 Hand sanitizer prevents Pathogen which involved in harmful diseases

Bacteria are a varied collection of unicellular prokaryotic creatures with a stiff cell that specifies their shape as coccoid (spherical), bacillary (rod-shaped), helical, or common. They can be found in practically every environment, including air, faeces, water, sewage, the human body, wounds, and other solid periphery. Some are good for the body, while others might be harmful (Young, 2006). The most prevalent illnesses to be concerned about are *Salmonella*, *Staphylococcus aureus*, and *E.coli* (Saharan et al., 2020). Hand washing dispels visible filth from hands and reduces the quantity of hazardous germs carried by humans, animals, or ingredients and passed on to food, such as *E. coli* and *Salmonella* (Shah et al., 2014). *Salmonella typhimurium* is a pathogenic bacterium that has several applications (Rosenberger et al., 2000). *S. typhimurium* causes salmonellosis, a frequent foodborne infection (Dar et al., 2017). Teaching proper hand washing procedures to one population and comparing the frequency of illnesses to a similar community that received no intervention was used in a study on strategies to prevent infectious disease. The number of diarrhea sufferers was reduced by 50%, according to the findings. In the intervention research, 95 percent of patients had diarrhea as a result of dirty hands. Handwashing with soap dramatically lowered the risk of diarrhea (Stebbins et al., 2011). *Escherichia coli* (*E.coli*), a Gram-negative bacteria widely found in human and animal guts, is a well-known diarrhea-causing pathogen. *E.coli* infection can result in a severe intestinal infection, resulting in diarrhea, abdominal pain, and a fever (Akbar and Anal, 2011). *S. aureus* is another pathogen that can be prevented by handwashing (*S.aureus*). When grown on a dense, enriched substrate, this facultative an aerobic pathogen appears as a golden colony because to the yellow pigment grape xanthin (Castro et al., 2016). *S.aureus* is a Gram-positive bacteria with the ability to multiply in blood vessels and tissue (Shell et al., 2005). The sigma B regulon, a transcription factor that governs the stress response of Gram-positive bacteria, is largely responsible for bacterial transition and adaptability (Claverys et al., 2006). The growth and pathogenicity of *Staphylococcus aureus* are both dependent on oxygen. The atmospheric oxygen level will drop once these bacteria are infected, and the *S.aureus* cytotoxins will rise in hypoxic settings (Arenas et al., 2013). *S aureus* is a bacterium that grows in a flora colony on mucous membranes (Von Eiff et al., 2001).

2.3 Importance of herbal hand sanitizer

Alcohol removes vital oils and sebum from the skin, which functions as a natural impediment against bacterial infection and cause protein to precipitate. As a result, when employed to wounds or raw peripheries, it not only rises the risk of harm, but also forms a coagulum in which bacteria can grow (Buzea et al., 2007). So, it is ineffective for disinfecting open lesions or abraded, irritated skin. These and other negative qualities, when combined, severely limit the alcohol-based antimicrobial product's quick effectiveness and raise the risk of disease expansion (Jumaa, 2005). The key advantages of using medicinal plants as a treatment for a variety of conditions are their safety, as well as their cost, efficacy, and accessibility (Debas et al., 2011). Plants contain a wide range of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids that have been proven to have antibacterial effects in vitro. In response to this need, an attempt was made to screen the classic literature for antimicrobial herbs. Plants are utilized in the creation of a variety of herb-based hand sanitizers due to their antibacterial properties (Gorlenko et al., 2020). Herbal hand sanitizer was tested using microorganism suspensions (Bacteria- E. coli, *Staphylococcus aureus*) and shown to be more effective than commercial synthetic hand sanitizer at reducing the number of microorganisms on the hand flora. These plant extracts' improved antibacterial activity and effectiveness can be used to make herbal hand sanitizers on a commercial basis (Surwase et al., 2021).

2.4 Natural herbs

In diverse human cultures across the world, more than 35,000 plant species are employed for medicinal purposes (Mukherjee and Wahile, 2006). Nature has produced healing compounds for thousands of years, dating back to the beginning of time. Medicinal plant usage, especially in traditional medicine, is now generally acknowledged and recognised as a valid profession (Pal and Shukla, 2003). Bioactive compounds derived from medicinal plants have been shown to have physiological action. It also facilitates pharmacology research, resulting in the production of a more powerful drug with lesser toxicity (Farombi, 2003). Medicinal plants may be used for more than simply curing illnesses; they can also be used to maintain good health (Petrovska, 2012). Herbal medicines are widely utilized in many parts of the world because they are widely available, culturally acceptable, have higher compatibility and adaption to human body physiology, and have less side effects (Kumari et al., 2013). In pursuance of World Health Organization (WHO), a medical plant is any plant that includes compounds in one or more of its organs that may be used to create effective drugs (Doughari, 2012). Secondary plant metabolites are complex chemical compounds found in one or more of these plants with different compositions (Figueiredo et al., 2008).

2.4.1 Neem

Neem (Azadirachta indica), sometimes called as Indian lilac, belongs to the mahogany family Meliaceae. It grows in India, Nepal, Pakistan, Bangladesh, Sri Lanka, and the Maldives, as well as other tropical and semi-tropical areas across the Indian subcontinent (Singla and Saini, 2019). Neem is used in traditional medicine in Indian culture as a source of many medicinal chemicals, and it grows well in tropical climates. Its twigs are commonly used as a chewing stick in the Indian subcontinent (Bhowmik et al., 2010). Nimbin, nimbidin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol and amino acid, 7-desacetyl-7-benzoylazadiradione, 17-hydroxyazadiradione, and nimbiol are all invented in the leaves. Nimbidin (the major active antibacterial element), nimbin and nimbinin are stable chemicals and found in substantial numbers (Kumari et al., 2020). The first polyphenolic flavonoids isolated from fresh neem leaves were quercetin and ß-sitosterol, which were known to have antifungal and antibacterial properties (Mahmoud et al., 2011). Previous study has showed that neem contains active chemicals with a wide range of medicinal properties (Shareef and Akhtar, 2018). Neem leaves are antimicrobial and can be used to reduce bacterial pollution in the air in a household setting (Raut et al., 2014). During the separation of nimbin, the first bitter chemical discovered from neem oil, more than 135 compounds were extracted from various portions of the neem tree (Brahmachari et al., 2004). Boiled neem leaf water is an antiseptic that may be used to treat wounds, reduce swelling, and relieve skin disorders (Sharma et al., 2009). Skin problems, good hair, enhanced l function of liver, detoxification of blood, antidiabetic, anti-inflammatory, antiviral, anticarcinogenic, immune-modulatory, and more are all treated with *Azadirachta indica*. The immunological response of Balb-c mice to sheep red blood cells was improved in vivo by aqueous stem bark extract (Kumar et al., 2019).

2.4.1.1 Scientific classification of Neem

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Sapindales

Family: Meliaceae

Genus: *Azadirachta*

Species: *A. indica*

Binomial Name: *Azadirachta indica*

2.4.2 Tulsi

The culinary plant Ocimum sanctum is known as basil (tulsi) (Gaddamwar *et al.*, 2020). Basil is native to India and other tropical Asian regions, where it has been cultivated for over 5,000 years (Shah *et al.*, 2018)**.** The Lamiaceae family contains the aromatic plant *ocimum sanctum* (Mittal *et al.*, 2018). *Ocimum sanctum* has been shown to have considerable anti-stress properties in the Ayurvedic School of medicine. Different parts of the plant can be applied to treat a variety of malady. (Kulkarni and Adavirao, 2018). It's a 30–60cm tall erect, heavily branched subshrub with hairy stems and simple opposite green or purple leaves with a strong perfumed smell. Petioles are present, and the leaves are oval, up to 5cm long, and somewhat serrated. It has long been regarded as one of the most valuable and holistic plants used in Indian traditional medicine, and practically every part of the plant has been discovered to have medicinal effects (Singh *et al*., 2010). Extracts of the plant have been demonstrated to possess physiologically active ingredients that are insecticidal, nematicidal, fungistatic, or antibiotic, and are utilized in traditional remedies (Grauso *et al.,* 2020). Holy basil has a camphoraceous scent and a strong aniselike, somewhat musky and lemony flavor. (Wangcharoen and Morasuk, 2007).Tulsi is used in traditional medicine in a number of ways; aqueous extracts from the leaves (fresh or powdered) are used in herbal teas or combined with other herbs or honey to increase therapeutic efficiency. Poisoning, stomachaches, common colds, headaches, malaria, inflammation, and heart disease are all traditional applications for Tulsi aqueous preparations. (Choudhury *et al.*, 2012).

2.4.2.1 Scientific classification of Tulsi

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: O.sanctum

Binomial name: *Ocimum sanctum* L.

2.4.3 Eucalyptus

Eucalyptus is a genus of trees belonging to the Myrtaceae family. The majority of the over 700 species in this genus are native to Australia. New Guinea, Indonesia, and the Phillipines all have a tiny population. Eucalyptus trees may be found in practically every part of Australia. They've also made their way into drier subtropical and tropical regions as far as Africa, the Middle East, India, the United States, and South America. These trees are deliberated invasive in many of these places (Grattapaglia *et al*., 2012). In certain locations, they are treasured for their economic applications, while in others, they are prized for their aesthetic value. Eucalyptus trees are prized for their timber, and certain species are also good sources of proteins, tannins, gum, and colors, but their most important commodity is eucalyptus oil, which can be extracted easily from their leaves (Cock *et al.*, 2009). This plant is high in polyphenols and terpenoids, with Eucalyptol or Cineole as the base composition (70 to 80 mg/ml) of the leaves (Behbahani *et al*., 2013). Essential oils from some Eucalyptus species (such as Eucalyptus pulverulenta) contain up to 90% cineol (Sefidkon *et al*., 2010). Eucalyptus oil is found in cleaning and deodorizing products, as well as cough medications and decongestants (Cock, 2009). Eucalyptus oil has insect repulsive features and is used in a variety of cosmetics. Traditional bush medicines made from Australian Eucalyptus species have also been used by Australian Aborigines. Various species have been reported to be used to make antiseptic washes (Batish *et al*., 2008). Microbiologists, horticulturists, global development researchers, and environmentalists have been drawn to some eucalyptus species because of desirable characteristics such as being fast-growing sources of wood, producing oil that can be used for cleaning and as a natural insecticide and antimicrobial activity (Singla and Saini, 2019).

2.4.3.1 Scientific classification of Eucalyptus

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Orders: Myrtales

Family: Myrtaceae

Genus: *Eucalyptus*

Species: *E. globulus*

Binomial name: *Eucalyptus globulus*

2.4.4 Mahogany

Swietenia macrophylla (Family: Meliaceae), sometimes known as big leaf mahogany, is a tropical wood tree native to Central America (Morris *et al*., 2000). S. macrophylla may be found in open rain forests, semideciduous forests, and deciduous forests (which lose some or all of their leaves during the dry season) (Daubenmire, 1972). S. macrophylla has been widely employed in ordinary people's communities all across the world, particularly in places where this plant is abundant. *Swietenia macrophylla* can be used to treat wound infections and skin problems (Eid *et al*., 2013) Swietenia macrophylla has been used as an antibacterial, antifungal, and antiplasmodial agent in various studies (Dharmalingam *et al*., 2012). There have also been several studies on the antifungal and antimalarial features of limonoids, a key component of Swietenia macrophylla (Moghadamtousi *et al*., 2013). Based on traditional use, herbs are chosen and blended to support organ systems responsible for detoxification and immunological function, as well as to prevent microbial development in various sections of the body system. Phytoconstituents such as flavonoids, alkaloids, tannins, and triterpenoids, which are largely made up of triterpenoids and limonoids, are abundant in many medicinal plants, including S. macrophylla (Mehrandish *et al*., 2019). S. macrophylla's pharmacological activities are mostly caused by the presence of terpenoids and limonoids. Similarly, two S. macrophylla limonoids, 2 hydroxy 3-swietenolide and 2 hydroxy-3-O-tigloylswietenolide, have been demonstrated to exhibit antibacterial efficacy against eight MDR bacterial strains (Eid *et al*., 2013).

2.4.4.1 Scientific classification of Mahogany

Domain: Eukaryota

Kingdom: Plantae

Phylum: Anthophyta

Class: Dicotyledoneae

Order: Sapindales

Family: Meliaceae

Genus: *Swietenia*

Species: *S. macrophylla*

Binomial name: *Swietenia macrophylla*

2.4.5 Ash gourd

Benincasa hispida, which is generally called ash gourd, winter melon, winter gourd, wax gourd etc. belongs to the family Cucurbitaceae (Rajalakshmi, 2018). It is a exoteric vegetable crop, particularly among Asian communities for nutritional as well as medicinal purposes (Pieroni *et al*., 2007). In most cases he Cucurbitaceae family is distributed around the tropical regions (Xu and Chang, 2017). According to scientific sources, B. hispida contains a variety of vital nutrients such as vitamins, natural sugars, amino acids, organic acids, and mineral elements (Zaini et al., 2011). *Benincasa hispida* leaf contains alkaloids, flavonoids, steroids (Nadhiya et al., 2014). Pharmacological studies revealed that the plant has a variety of pharmacological activities, including anti-oxidant, anti-inflammatory, analgesic, anti-asthmatic, diuretic, nephron protective, antidiabetic, hypolipidemic, and antimicrobial effects, as well as central nervous effects (anxiolytic, muscle relaxant, antidepressant, in the treatment of Alzheimer's disease, and to minimize opiates withdrawal symptoms) (Jadoon et al., 2015).

2.4.5.1 Scientific classification of Ash gourd

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Sub class: Dilleniidae

Orders: violales

Family: Cucurbitaceae

Genus: *Benincasa*

Species: *B.hispida*

Binomial name: *Benincasa hispida*

2.4.6 Meadow grass

Cynodon dactylon is a perennial grass of the Poaceae family that grows all over the world. It grows in tropical, sub-tropical, and even semi-arid regions practically everywhere (Abdullah *et al*., 2012). In India, Cynodon dactylon is one of the most frequent weeds. Durba (Bengali), garikoihallu (Kanarese), durua (Marathi), durua or haritali (Sanskrit), arugampullu (Tamil), garikagoddi (Telugu), and dhubkhabbal (Punjabi) are some of the other prevalent names for it (Garg and Khosa, 2008). It is a tough, perennial grass that may be found all over the world, but is most common in warm temperate and tropical climates (Lewandowski et al., 2003). It's a creeping grass that's strong, drought-tolerant, light green in color, gritty in texture, and grows quickly. It comes in short cylindrical bits ranging in length from 3 to 20 mm and diameter from 2 to 3 or sometimes 4 mm (Dande et al., 2012). More than 40,000 plant species are employed as medicinal herbs in traditional and ethnomedical practices across the world (Jadid et al., 2020). In India, Cynodon Dactylon is a traditional medication that is well-known for minor treatments (Chandel and Kumar, 2015). C.dactylon is traditionally used as a rejuvenator and for wound healing (Mangathayaru et al., 2009). The astringent juice of the plant is administered topically to new cuts and wounds (Singh et al., 2002). As secondary metabolites, plants create a wide number of organic elements such as alkaloids, flavonoids, glycosides, tannins, terpenoids, and phenolics, which are employed as defense mechanisms against pathogens (Abdullah et al., 2012). The phytochemical contents of C. dactylon were discussed, as well as its antibacterial effectiveness against certain common microorganisms such as *E. coli,* (*Staphylococcus aureus*, and *Pseudomonas aeruginosa*, all of which have been linked to nosocomial infections Savadi, 2020).

2.4.6.1 Scientific classification of Meadow grass

Domain: Eukaryota

Kingdom: Plantae

Division: Tracheophyta

Class: Magnoliopsida

Orders: Poales

Family: Poaceae

Genus: *Cynodon*

Species: *C.dactylon*

Binomial name: *Cynodon dactylon*

2.4.7 Tamarind

Tamarindus indicia L. is a member of the Caesalpiniaceae family, which is the third biggest flowering plant family, with 727 genera and 19, 327 species (Khanzada et al., 2008). T. indica is farmed in the subtropics and semi-arid tropics, where it has a wide geographical range. Tamarind grows wild in Asia from Burma to Afghanistan, up to an altitude of about 500 meters. It is found across the Indian subcontinent, particularly in the southern and central regions (which share the wet and semi-arid climatic features of tropical regions) (Meher et al., 2014). Tamarind has been utilized as a medicinal plant for ages; the most useful portion of the plant is the fruit, which has been described as therapeutic in numerous pharmacopoeias. Other plant sections, on the other hand, have received less attention. The presence of polyhydroxylated chemicals, many of which are flavonolic in origin, has been linked to hepatoprotective action in the leaves (Escalona et al., 1995; Joyeux et al., 1995). Protein, fat, fiber, and vitamins including thiamine, riboflavin, niacin, ascorbic acid, and B-carotene are all abundant in leaves (Abdallah and Muhammad, 2018). Tamarind leaves have a long history of ethnobotanical usage due to their antibacreial, antifungal, antiseptic effects in Latin America, particularly in Mexico, Puerto Rico, and Trinidad and Tobago, as well as in other continents such as Asia and Africa (Escalona-Arranz et al., 2010). Tamarind leaves help with inflammation, tumors, ringworm, blood illnesses, small pox, ophthalmia and other eye problems, earaches, and snake bites (Basu et al., 2006). Antibacterial efficacay of ethanolic extracts of *Tamarindus indica* leaf and stem extracts against certain gram-negative bacteria (Nwodo, 2011). *Tamarindus indica* leaf extracts, both hydroalcoholic and aqueous, have antibacterial action against gram positive and negative bacteria such as *Staphylococcus aureus, Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Escalona-Arranz et al., 2010).

2.4.7.1 Scientific classification of Tamarind

Domain: Eukaryota

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Fabales

Family: Fabaceae

Genus: *Tamarindus*

Species: *T.indica*

Binomial name: *Tamarindus indica*

2.4.8 Guava

The tropic tree *Psidium guajava* (often known as guava) is extensively planted for its fruit. It is a member of the Magnoliophyta phylum, the Magnoliopsida class, and the Myrtaceae family. Guava in English, guayabo in Spanish, goyaveandgoyavier in French, guyabaorgoeajaab in Dutch, goiaba and goaibeira in Portuguese, and jambubatu in Malaya are all frequent names for the plant. In Mexico and America, the terms pichi, posh, and enandi are often used (Naseer et al., 2018). Guava trees, which are native to tropical regions from southern Mexico to northern South America, have been effectively planted in many other tropical and subtropical nations, allowing for producing globally (Singh, 2005). Guava leaf extract includes flavonoids, mostly quercetin derivatives, which are hydrolyzed in the body and converted to the aglycone quercetin, which is responsible for the leaves' spasmolytic effect. Quercetin has numerous pharmacologic functions, including inhibiting intestinal motility, lowering abdominal capillary permeability, and possessing dose-dependent antioxidant, anti-inflammatory, antiviral, and anticancer characteristics. (Metwally et al., 2010). Guava leaf extracts have excellent antibacterial activity and can stop S. aureus from growing (Hoque et al., 2007). The essential oil in guava leaves contains cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a variety of other fixed compounds. P. guajava methanolic extracts from plant leaves and bark exhibit significant antibacterial activity. Bacillus and Salmonella bacteria can be inhibited by these extracts (Biswas et al., 2013). Polyphenolic compounds such as protocatechuic, ferulic, ascorbic, gallic, and caffeic acids, as well as quercetin, have been connected to the biological benefits of guava (Laily et al., 2015).

2.4.8.1 Scientific classification of Guava

Domain: Eukaryota

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Myrtales

Family: Myrtaceae

Genus: *Pisidium*

Species: *P. guajava*

Binomial name: *Psidium guajava*

2.4.9 Gulancho lata

Tinospora sinensis, also known as "Sudarsana" in Sanskrit, "Giloy" in Hindi, and "Hoguni lota" in Assamese, is a plant with a wide variety of medicinal characteristics that is used by several native tribes to cure disorders such as fever, urinary infection, jaundice, diabetes, and more (Das et al., 2020). *Tinospora sinensis* is a species of Tinospora (Lour.) Merr is a member of the Menispermaceae family, and the plant's stem is utilized in medicine. The plant grows wild in both woodlands and plains over most of India. The plant is claimed to be used to cure piles and ulcerated wounds, as well as to prepare liver-healing baths. Cooked roots are used to cure fever. Fresh leaves and stems are used to treat chronic rheumatism and as a muscle relaxant (Gairola et al., 2014). Anti-inflammatory properties have been found for Tinospora sinensis. Antiinflammatory, analgesic, hypoglycemic, hepatoprotective, cytotoxic, antiallergy, antioxidant, antimalarial, antiscabies, and antibacterial properties have all been associated to it (Castillo et al., 2018). *T. sinensis* has previously been shown to have antimicrobial action. Antimicrobial action against Staphylococcus aureus is found in its roots and leaves (Cushnie et al., 2005).

2.4.9.1 Scientific classification of Lata

Domain: Eukaryota
Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Ranunculales
Family: Menispermaceae
Genus: *Tinospora*
Species: *T.sinensis*
Binomial name: *Tinospora sinensis*

2.5 Phytochemicals

Phytochemicals (from the Greek word phyto, meaning "plant") are physiologically active, naturally occurring chemical compounds found in plants that, in addition to macronutrients and micronutrients, provide health benefits to humans (Saxena et al., 2013). They protect plants from disease and damage while also improving their color, smell, and flavor. Phytochemicals are plant compounds that protect plant cells from environmental threats such as pollution, stress, dehydration, UV exposure, and pathogenic infection (Oz and Kafkas, 2017). Medicinal plants include a range of phytochemicals or secondary metabolites that can be used alone, in combination, or synergistically to benefit health (Briskin, 2000). Plant phytochemicals include alkaloids, flavonoids, tannins, terpenoids, glycosides, saponins, and anthraquinones (Ajayi et al., 2011).

2.5.1 Alkaloids

Alkaloids are the most frequent sort of secondary plant metabolite, and they're primarily nitrogen bases made by replacing one or more hydrogen atoms in the peptidic ring with various radicals, the majority of which include oxygen. These nitrogenous chemicals are commonly utilized as medications, psychostimulants, narcotics, and poisons in plant defense against enteric pathogenic organisms due to their well-known biologic activities (Hao, 2009).

2.5.2 Glycosides

Glycosides are the condensation products of sugars containing a variety of organic hydroxyl or thiol compounds, with the hemiacetal moiety of the carbohydrate playing a minimal role in the condensation process. Glycosides are solely bitter compounds found in the Genitiaceae family of plants. They have nothing in common chemically, yet they both have a very bitter flavor. By acting on the gustatory nerves, bitters promote the flow of saliva and stomach fluids. Bitter principles have a lactone group, which might be diterpene lactones (as in andrographolide) or triterpenoids (as in andrographolide) (as in amarogentin). Several bitter ingredients are used as astringents, antiprotozoans, or to reduce thyroxine and metabolism due to the presence of tannic acid. Anthracene glycosides (purgative and for the treatment of skin diseases), chalcone glycoside (anticancer), amarogentin, gentiopicrin, rographolide, ailanthone, and polygalin are examples of anthracene glycosides (purgative and for the treatment of skin diseases), amarogentin, gentiopicrin, rographolide, ailanthone, and polygal (Adeosun et al., 2016).

2.5.3 Flavonoids

Flavonoids are polyphenols that may be found in a variety of plant species. They contain more than one benzene ring, and studies have indicated that they can behave as antioxidants or free radical scavengers (Kim and Lee, 2004). Flavans, which are parent compounds, are used to make the chemicals. Around 4,000 flavonoids have been identified, with some functioning as pigments in higher plants. Nearly 70% of plants contain flavonoids such as quercetin, kaempferol, and quercitrin. Other flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins, proanthocyanidins, calchones, catechins, and leucoanthocyanidins (Omojate Godstime et al., 2014).

2.5.4 Polyphenols

Phenolics, phenols, or polyphenolics (or polyphenol extracts) are chemical components found in abundance in plants' fruits as natural color pigments. Phenolics are primarily generated in plants by phenylalanine ammonia lyase from phenylalanine (PAL). They are required by plants and have a range of functions. Plant defense against viruses and herbivore predators may be the most important function, thus it's no surprise that they're employed to treat human pathogenic illnesses (Tovar et al., 2002).

2.5.5 Saponins

Saponins are chemicals that behave like soap in water, producing foam when shaken. An aglycone named sapogenin is generated during hydrolysis. Saponins are chemicals that behave like soap in water, producing foam when shaken. An aglycone named sapogenin is generated during hydrolysis. Saponins are particularly dangerous because they promote blood heamolysis and have been linked to animal poisoning. They have a bitter and caustic flavor, as well as irritate mucosal membranes (Omojate Godstime et al., 2014).

2.5.6 Tannins

Tannins have antibacterial properties owing to the presence of the phenolic group. Ayurvedic formulations based on tannin-rich plants have been utilized to treat enteric disorders such as diarrhea in Ayurveda (Omojate Godstime et al., 2014).

2.6 Nanoparticles

Due to the growing microbial resistance to metal ions, antibiotics, and the development of resistant strains, metallic nanoparticles are the most promising for all of the aforementioned purposes because they have remarkable antibacterial properties due to their large surface area to volume ratio, which is of interest to researchers (Ahmed et al., 2016). Plants have been seen to produce nanoparticles of gold, silver, copper, silicon, zinc, titanium, magnetite, and palladium. The catalytic, antibacterial, excellent conductivity, and chemical stability of colloid silver nanoparticles were all demonstrated. Silver nanoparticles are used in bio labeling, sensors, antimicrobials, catalysis, electronics, and other medical applications such as medication administration and illness detection (Begum et al., 2019). Organic nanoparticles and inorganic nanoparticles are the two primary categories of nanoparticles. Carbon nanoparticles (fullerenes) are organic nanoparticles, while magnetic nanoparticles, noble nanoparticles (gold and silver), and semiconductor nanoparticles are inorganic nanoparticles (titanium oxide and zinc oxide). Inorganic nanoparticles, in particular, have attracted interest due to their exceptional material characteristics and diverse uses. It is conveniently employed for chemical imaging pharmaceuticals, agents, and medications due to its nano size. Its diverse function is employed for cellular distribution since it is readily accessible, has a lot of versatility, and is biocompatible. This is also a good vehicle for delivering targeted medications and regulating drug release. (Grubbs, 2007).

2.6.1 Silver nanoparticles

Silver nanoparticles have attracted and required attention in the field of nanotechnology due to unique properties such as high conductivity, chemical stability, catalytic activity, surface enhanced Raman scattering, and antibacterial activity (Kaler et al., 2010). Among the nanoparticles used in the pharmaceutical industry, silver nanoparticles are one of the most important elements in nanomedicine. Antibacterial silver nanoparticles (Ag NPs) have been used to treat bacterial skin infections as topical antibacterial therapies (Hamad et al., 2020). The catalytic activity of the produced AgNPs in the degradation of 4-nitrophenol by $NaBH_4$ was also studied. Antibacterial activity of AgNPs was also evaluated against harmful bacterial strains such as E. coli, Pseudomonas aeruginosa, and Staphylococcus aureus (Naraginti and Sivakumar, 2014).

2.7 Olive oil as moisturizing and hydrating agent

Olive oil is a liquid fat made from the pressing of olives. Olive oil has been used for skin treatment since ancient Egypt. The fatty acid ratio of olive oil (palmitic acid, oleic acid, linoleic acid, linolenic acid) may be specified, with oleic acid being the most important component (Karagounis et al., 2019). Triacylglycerols make up the majority of olive oil (98–99 percent). Triacylglycerol (TGA) are a category of glycerol esters that contain a variety of fatty acids. Monounsaturated oleic acid (up to 83 percent w/w) is the most abundant fatty acid in olive oil TGAs. The remaining TGAs in olive oil are palmitic acid, linoleic acid, stearic acid, and palmitoleic acid. Phytosterols, squalene, tocopherols, phenolic compounds, terpenic acid derivatives, and other lipophilic or amphiphilic microconstituents can all be found in virgin olive oil. Polyphenol concentration varies between 50 and 1000 mg/kg in olive oil. In actuality, it is determined by agronomic elements such as olive maturity, extraction technique, and storage or packaging methods, as well as other considerations (Gorzynik-Debicka et al., 2018). Moisturizers are items that are applied to the skin (mostly emulsions, lotions, and creams) to improve moisture in both healthy and dry, rough skin. (Lodén, 2003). In cosmetics and medicines, olive oil is becoming increasingly commonly utilized. Olive oil has long been known for its medicinal and health-promoting benefits. Olive oil is rich in critical components like squalene, phytosterol, and tocopherol, as well as other essential vitamins (A and E) and fatty acids like oleic, linoleic, and linolenic acids, all of which soothe and protect the skin. Olive oil's attractive biological qualities are attributed to the presence of minor components such as squalene and phytosterols, antioxidant chemicals such as tocopherols, and especially phenols (Smaoui et al., 2012). The goal of moisturizing agent-containing formulations is to achieve an appropriate balance between water and skin lipids by ensuring the skin's water content in a variety of methods. Again, olive oil includes squalene and vitamin E, both of which are beneficial to skin care products. Vitamin E enhances skin hydration and water-binding ability, while squalene hydrates and protects the skin. (Pavlou et al., 2021).

2.8 Glycerin as humectant, emollient and moisturizing agent

Glycerin is a polyhydric alcohol with the chemical formula $C_3H_8O_3$ as a general rule. Glycerin (also known as glycerol) is a simple triol, meaning it has three hydroxyl groups (Becker et al., 2019). Glycerin is a transparent, syrupy liquid with a sweet taste. It can be crystallized, but this is uncommon due to its proclivity for super cooling and the significant influence of tiny amounts of water in lowering the freezing point (Wolfe and Bryant, 2001). Glycerin has comparable solvent characteristics to water and simple aliphatic alcohols. Water, methanol, ethanol, and the propanol, butanol, and pentanol isomers are all entirely miscible with this chemical (Marks, 1967). Glycerin is found in all animals and plant materials in the form of glycerides in fats and oils, or as lipids in intracellular spaces. While the ingredients are the same, there are two types of glycerin: natural glycerin taken from plants and animals, and synthetic glycerin created from nontriglyceride sources. In cosmetics, glycerin is used as a denaturant, hair conditioner, humectant, oral care agent, oral health-care drug, skin protectant, skin conditioner—humectant, and viscosity-decreasing agent. (Becker et al., 2019). In a controlled, randomized, double-blind trial, it was shown that using an emollient combination (myristyl alcohol, glycerol, dexpanthenol, levomenol, and lanolin alcohol) reduced contact dermatitis (Kampf et al., 2005). The right selection of emollients, or their combination, is critical to the product's ability to moisturize the skin (Kraft and Lynde, 2005). Glycerin is an emollient that hydrates and softens the skin while also reducing irritation and flaking caused by harsh chemicals (Lodén, 2003). Glycerine boosts hydrogen bonding, making ethanol evaporation more difficult. As a result, only the bare minimum is necessary (Ochoa-Gómez et al., 2012). Glycerin has been demonstrated to be an effective humectant, and studies have indicated that using humectant-containing moisturizers can enhance the barrier against skin irritants and hasten the regeneration of injured skin. Dry skin may have a larger proportion of solid lipids, such as eicosapentaenoic (EPA), docosahexaenoic (DHA), and stearidonic acid, and glycerin may help retain the lipids in a liquid crystalline condition at low relative humidity (Loden, 2005). The potential of glycerin to treat dry skin complaints varies depending on concentration (Fernandez-Dıaz et al., 2001).

2.9 Honey as antimicrobial agent, moisturizing and humectant agent

Honey has been one of the most widely utilized substances in human history, and its skin-beneficial properties have long been known. Honey has been utilized widely in toilet preparations and early skin cures since ancient times. Nomadic tribes of the Tasian civilization (about 4500 BC) blended malachite, copper, spar, oil, fat, and honey for eye cosmetics, according to archeological documents from the predynastic period of Upper Egypt. Honey was also used for skin treatment in the ancient world, according to a Sumerian tablet from circa 3000 BC and the Egyptian EbersPapyrus (around 1500 BC). Honey is likely the oldest skin-care product in use today, as evidenced by several traditional usage in various nations. In East Asia, Japanese ladies are said to use honey on a regular basis to keep their hands wrinkle-free. Antiseptic actions have been the subject of a lot of research on honey's wound healing qualities. Honey is hygroscopic, antibacterial, and antifungal, and it nourishes the skin while also helping to control the moderately acid pH of the upper protective layer. It may be used as a natural component in a wide range of moisturizing products due to its humectant properties, and its cleansing properties can be used in skin soaps, bath and shower products, face creams, and lotions (Burlando and Cornara, 2013). Honey also has demulcent and anti-irritant effects, making it ideal for newborns and other people who have sensitive skin (Foster and Duke, 2000). Similarly, moisturizing and anti-irritant properties in sun care and sun screen products help to mitigate the effects of radiation-blocking compounds (Burlando and Cornara, 2013). Honey has been shown to have antibacterial properties. Honey is effective against a wide range of microorganisms. (According to Basson and Grobler, 2008). Honey alcohol extracts inhibit a wide range of bacterial species, including aerobes and anaerobes, Gram positives and Gram negatives (Abeshu and Geleta, 2016).

2.10 Ingredients used in Market ABHS (Alcohol Based Hand Sanitizer)

Humectants, thickeners, and perfumes are the most widely used constituents in commercial ABHS formulations (Nyamweya and Abuga, 2020). Thickeners are used to improve viscosity and make ABHS simpler to apply by minimizing spillage and making them easier to handle. Acryl acid and its derivatives, such as carbomers, are the most common polymers. Antimicrobial agents such as chlorhexidine have been mixed with alcohol in several ABHS products. Its added advantage, however, has been questioned. Such combinations are unusual, and in certain countries, they may be limited to ABHS utilized in health-care contexts (Abuga and Nyamweya, 2021). The presence of hydrogen peroxide, a powerful oxidizing agent, may result in additional possible interactions. The two WHO-recommended versions include hydrogen peroxide (H_2O_2) (Mileto et al., 2021). Because alcohols are sporicidal, hydrogen peroxide is employed to kill any bacterial spores present (Russell, 1990). This formulation has a few challenges and concerns in terms of fire hazard and skin toxicity because of the high alcohol content (Jing et al., 2020). The ABHS used in the United States lists tocopherol, perfumes, propylene glycol, benzoates, and cetyl stearyl alcohol as probable allergens (Abuga and Nyamweya, 2021). In personal care items, fragrances, in particular, are a common source of contact allergies (Ortiz and Yiannias, 2004). While certain aroma compounds are moderate sensitizers on their own, oxidation of parent compounds can lead to the production of powerful allergies. (Karlberg et al., 2008).

Chapter 3: Materials and Method

The study was conducted from December 2020 to January 2022 in Chattogram Veterinary and Animal Sciences University (CVASU) at the laboratory of Applied Chemistry and Chemical Technology, Physiology, Biochemistry and Pharmacology Department. *E.coli and Salmonella* spp. and *Staphylococcus* bacteria have used in this experiment. *E.coli and Salmonella* spp. were collected from the Research Lab. *Staphylococcus aureus* was collected from Microbiology lab.

3.1 Experimental design

The study was undertaken to develop alcohol based herbal hand sanitizer with honey, olive oil, glycerin, hydrogen peroxide, and ten types of herbs extract such as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Gulancho lata and Guava. In this study another two types of hand sanitizers are formulated, they are: Neem nanoparticle hand sanitizer, Eucalyptus nanoparticle hand sanitizer.

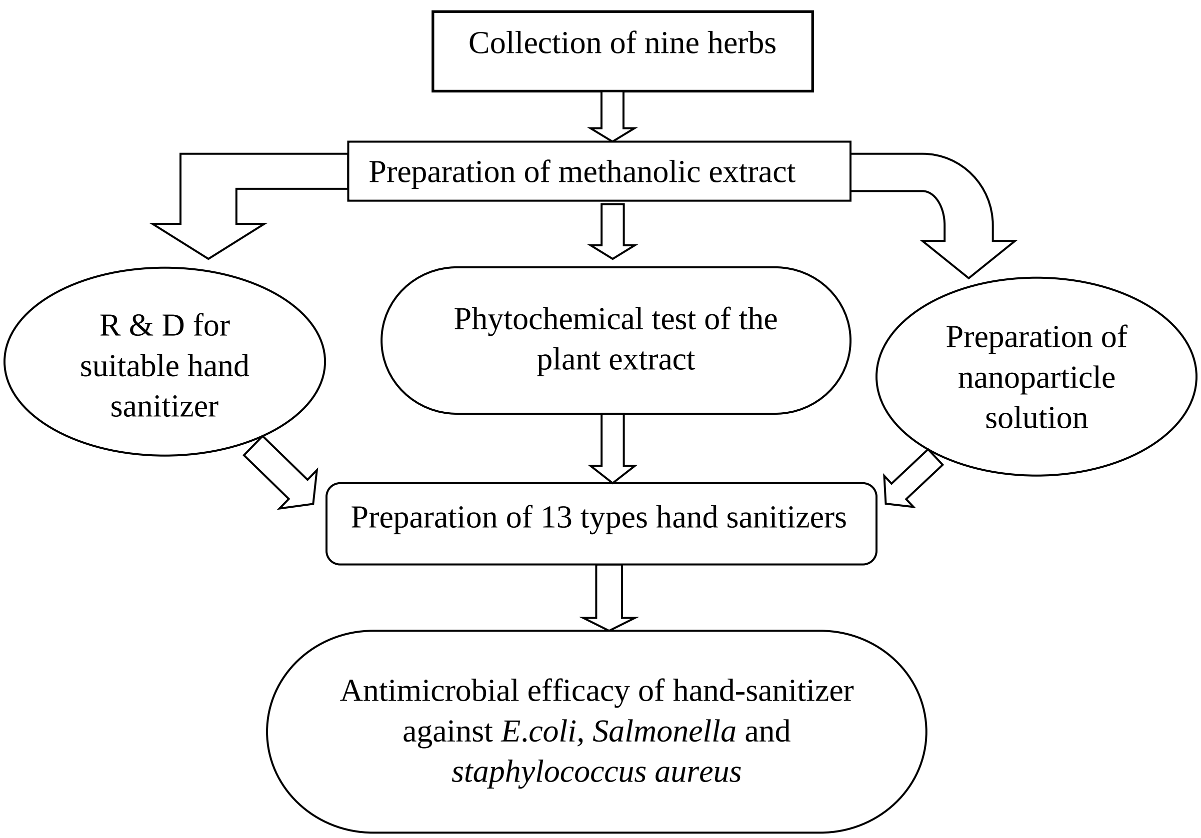

Figure 3.1 Overview of Experimental Design

3.2 Chemicals and reagents used in this study

Isopropyl alcohol (IPA)

It is an important substance in sanitizing process because it can kill the surface bacteria. So it is used in this study as an antibacterial agent.

Glycerin

It acts as the best emollient, moisturizer and lubricant to restrain skin dryness in personal care products.

Distilled water

It was used as a solvent.

Honey

It serves as an antimicrobial and hydrating agent. So, it prevents the skin from excessive dryness. It also used as a thickening agent.

Olive oil

It is a Moisturizing agent and essential for skin health.

Hydrogen peroxide:

It is used as an antimicrobial and oxidizing agent.

Lemon extract:

It was used in this hand sanitizer for fragrance.

Sepnil (Market brand hand sanitizer):

It is a renowned market brand hand sanitizer. The chemical components of this product are 70% ethanol, carbomer, glycerin, polyethylene glycol, TEA (Triethanolamine), Aqua, perfume. It was used in this study to compare the formulated hand sanitizer with this according to microbial test.

Hexisol

It is a hand sanitizer which consist of 0.5% chlorhexidine gluconate and 70% Iso propyl alcohol. It was used in this study to compare the formulated hand sanitizer with this according to microbial test.

Plant extract

There are 9 herbs (Tulsi, Neem, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata) are used in this study. These herbs are rich in secondary metabolites such as alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic compound, phytosterol, etc. These secondary metabolites have antimicrobial property.

Table 3.1: Herbs used in this study

Scientific Name	Local Name	Common name	Family	Plant part used
Azadirachta indica	Neem	Neem	Meliacea	Leaves
Ocimum sanctum	Tulsi	Basil	Lamiacea	Whole herb
Eucalyptus globulus	Eucalyptus	Eucalyptus	Myrtaceae	Leaves
Swietenia Macrophylla	Mahogany	Mahogany	Meliaceae	Leaves
Benincasa hispida	Chal kumra	Ash gourd	Cucubitacea	Leaves
Cynodon dactylon	Durba ghash	Meadow grass	Poaceae	Leaves
Tamarindus indica	Tetul	Tamarind	Fabaceae	Leaves
Pisidium guajava	Peyara	Guava	Myrtaceae	Leaves
Tinospora sinensis	Lata	Gulancho lata	Menispermaceae	Whole herb

Methanol

It was used as a solvent during plant extract preparation.

Silver nitrate

It was used for preparation of nanoparticle solution.

Mercuric chloride

It was used in Mayer’s test for detecting Alkaloid in the crude plant sample.

Potassium iodide

It was used in the Mayer’s test for detecting Alkaloid in the crude plant sample. It was also used for indicating the presence of Alkaloid in the Wagner’s test.

Iodine

It was used in Wagner’s test for detecting Alkaloids.

Sodium hydroxide

Sodium hydroxide solution was used in the alkaline reagent test for indicating the presence of Flavonoids.

Lead acetate

It was used in the Lead acetate test to detect the presence of Tannins.

Ferric chloride

It was used in the ferric chloride test for detecting the presence of Phenolic compound. It was also used in the Cardiac glycosides test for detecting the presence of Glycosides.

Glacial acetic acid

It was used in the Cardiac glycosides test for the detection of glycosides which was present in the plant sample.

Sulfuric acid

It was used in the Cardiac glycosides test for the detection of glycosides. It was also used in Molisch's test to indicate the presence of Carbohydrate.

Molisch's reagent

The compositions of Molisch's reagent are α-naphthol and 95% alcohol. It was used in the Molisch's test for indicating the presence of Carbohydrate.

Sodium bicarbonate

It was used to indicating the presence of acidic compound in the plant sample.

MacConkey and EMB agar

These agars were used for the isolating and identifying *E. coli* and *salmonella* in this study.

Mueller-Hinton agar

It was used for sub-culturing the *E. coli* and *Salmonella* which were preserved previously.

Brain heart infusion (BHI) broth

It is used for preparation and preservation of bacterial colonies.

Sulfuric acid and Barium chloride

These were used for McFarland standard solution preparation.

3.3 Instruments and apparatus

Conical flask, measuring cylinder, analytical balance, grinder, dryer, what man No.1 filter paper, rotary evaporator, pipette, micro pipette, digital pH meter, hot air oven, autoclave, incubator, electric cooker, vortex machine, foil paper, petri dish, Eppendorf tube were used in this study.

3.4 Extract Preparation

3.4.1 Collection of plant materials

The leaves of 9 different plants were collected for the preparation of sanitizer from in and around different sides of Chattogram. The plants selected on the basis of its potent antimicrobial activity. After collection of the sample, they were packed with polythene and transferred to Chattogram Veterinary and Animal Sciences University.

3.4.2 Drying and Grinding

Collected plant leaves were thoroughly cleaned by washing and discarding of all the unwanted materials and aged leaves were discarded. The leaves were sun dried for 10 days for removing moisture. The dried leaves were milled to fine powder with a blender. Then the powder was sieved for obtaining fine dust, and preserved the powder into an air tight plastic bag in the dark until used.

3.4.3 Preparation of methanolic extracts

To prepare methanolic extract of nine different plants 10 g of each plant powder were taken into nine conical flasks with 100 ml 90% methanol solution. Then the mixture was stirred for 10 minutes. Then heated the solution on water bath at 60°C for 1 hour. Further the mixture of each plant solution was filtered through Whatman no.1 filter paper separately (Wani et al., 2013). Then the filtrates were concentrated by extraction process using a rotary vacuum evaporator. During extraction process there was 160 to 170 rpm in the machine. The temperature of the machine is 50 to 55°C. After the evaporation the extracts were preserved in a vacutainer tube and stored in a refrigerator (4°C).

3.5 Phytochemical activity test

Phytochemicals present in the leaf extract s were screened as per standard protocols for phytochemical constituents such as alkaloids, glycosides, tannins, saponins, phenols, flavonoids, Steroids and Terpenes, Phenolic Compound, Glycosides etc. Phytochemical examinations were carried out for all the extracts of Tulsi, Neem, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata) as per the standard methods (Sofowora, 1996).

3.5.1 Detection of Alkaloids

Mayer's Test

1.36 g mercuric chloride was dissolved in 60 ml distilled water to make Mayer's reagent. It put a 5 g potassium iodide solution in 20 ml distilled water. Both solutions were combined and diluted in distilled water to a volume of 100 ml. A few drops of the reagent listed above were applied to a little amount of the test residue. The presence of alkaloids was demonstrated by the production of a cream-colored precipitate.

Wagner's test

1.27 g iodine and 2 g potassium iodide were dissolved in 5 ml water and diluted to 100 ml with water. A reddish-brown precipitate appears when a few drops of this reagent are applied to the test residue, indicating the presence of alkaloids.

3.5.2 Detection of Flavonoids

Alkaline Reagent Test

Sodium hydroxide (NaOH) solution was used to treat the extracts. The presence of flavonoids is indicated by the production of a bright yellow hue that becomes colorless when dilute acid is added.

3.5.3 Detection of Saponins

Foam test

For a stable, persistent froth, 2g of the plant extract was combined with 10ml of distilled water and forcefully shaken. The presence of saponins is indicated by the development of foam.

3.5.4 Detection of Tannins

Lead acetate test

2mL of plant extract and 2mL of distilled water were mixed together. To this combined solution, 0.01g lead acetate was added and well mixed. The presence of tannins is indicated by the appearance of white turbidity and precipitate.

3.5.5 Detection of Phenolic Compound

Ferric chloride test

2ml plant extract was added to water and heated to 45-50°C. Then 2 mL of $FeCl_3$ at 0.3 percent were added. The presence of phenols is indicated by the production of a green or blue tint.

3.5.6 Detection of Glycosides

Cardiac Glycosides (Killer-Killani test):

The test residue was treated with 1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of concentrated sulfuric acid, and the development of a reddish-brown hue at the intersection of two layers was seen. In the presence of glycosides, the top layer became blue green.

3.5.7 Detection of Carbohydrates

Molisch's test:

10 g of -naphthol was dissolved in 100 ml of 95 percent alcohol to make Molisch's reagent. 2 drops of Molisch's reagent were added to a few milligrams of test residue. 1 mL concentrated sulfuric acid was added to this solution from the slanted test tube side. In the presence of sugars, there was no reddish violet ring at the confluence of the two layers.

3.5.8 Detection of Acidic Compound

Sodium bicarbonate test

The production of effervescence was seen after adding sodium bicarbonate solution to the alcoholic extract.

3.6. Preparation of Silver nanoparticles by plant extract (AgNPs)

To begin, standard solutions were made by mixing 1 mL plant extracts with 99 mL deionized water and 0.5 mL emulsifier in water. The silver nanoparticles were then made by adding 1 mL of a 100 micro molar silver nitrate solution to 99mL of the standard solution. Nine different plant extracts were made and kept at room temperature until the hue changed to brown, indicating nanoparticles production. (Barnawi *et al.,* 2019).

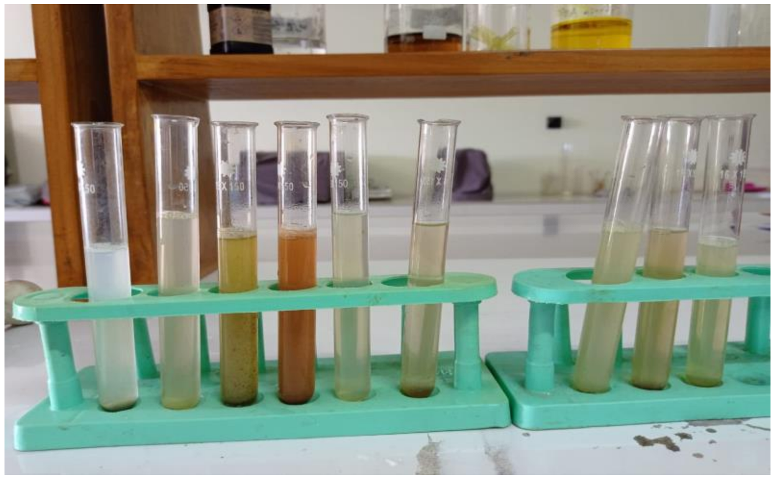

Figure 3.2: Silver nanoparticles solutions of plants

3.7 Research and development (R&D) for suitable hand sanitizer formulation

Our hands have gotten very dry as a result of our frequent usage of hand sanitizer. Hand sanitizer contains alcohol, which can dry out our skin. This can be remedied by applying a hand moisturizer and staying hydrated. Hand sanitizers containing polyunsaturated fatty acids, glycerin, and honey may be an effective hand softening. Olive oil may be used as a hand softener in most cases; however, it is not soluble at all concentrations. As a result, R&D was carried out to determine the appropriate concentrations of alcohol and olive oil at which they are soluble. The concentration of a sufficient amount of glycerin, honey, scent, water, and other substances used in hand sanitizer manufacturing was also determined through R&D.

3.8 Preparation of Thirteen types Alcohol based herbal hand sanitizer after R & D

3.8.1 Preparation of nine types of alcohol based herbal hand sanitizer

At first nine conical flask were taken. Then 80 ml of 75% Isopropyl alcohol (IPA) were taken in each of the nine conical flasks. Thereafter, 1.5 ml honey, 3ml olive oil and 2ml of 98% glycerin were mixed separately with IPA in nine conical flasks. After doing that stirred the solutions with a glass rod. Finally, 2% plant extract of each plant, 1·5 ml of 3% Hydrogen per oxide, 2ml lemon extract and 8ml distilled water were mixed with previous solutions respectively. Then slightly shook the finally prepared solutions.

Figure 3. 3: Formulated herbal hand sanitizers

Table 3.2: Final formulation of 9 type alcohol based herbal hand sanitizers

Elements	Value (ml)	Sources
IPA (75%)	80	WHO
Distilled Water	8	R&D
Glycerin (98%)	2	R&D
Honey	1.5	R&D
Olive oil	3	R&D
Plant extract (9 different plants)	2	R&D
Hydrogen Per Oxide (3%)	1.5	WHO
Lemon extract	2	R&D

3.8.2 Preparation of mix herbal hand sanitizer

First of all, 80 ml of 75% Isopropyl alcohol (IPA) was taken in a conical flask. Then I added 1.5% honey, 3% olive oil and 2% glycerin with IPA. After doing that stirred the solutions with a glass rod. Thereafter, I took 0·25 % plant extract of each nine plant. Thus, a total of 2.25% plant extract was taken in the previous solution. Then 1·2% Hydrogen per oxide, 2% lemon extract and 18·3% distilled water were added to the solution. Thereafter slightly shook the finally prepared solution

Table 3.3: Formulation of mix herbs hand sanitizer

Elements	Value (ml)	Sources
IPA (75%)	80	WHO
Distilled Water	8	R&D
Glycerin (98%)	2	R&D
Honey	1·25	R&D
Olive oil	3	R&D
Mix plant extract (9 different plants)	2·25	R&D
Hydrogen Per Oxide (3%)	1.5	WHO
Lemon extract	2	R&D

3.8.3 Preparation of blank hand sanitizer (without plant extract)

In blank sanitizers all ingredients were used except plant extract. At first, I took 80 ml of 75% Isopropyl alcohol (IPA) in a conical flask. Then I added 1.5ml honey, 3ml olive oil and 2ml of 98% glycerin with IPA. After doing that stirred the solutions with a glass rod. Then 1·5 ml of 3% H_2O_2, 2ml lemon extract and 10 ml distilled water were added to the previous solution. Thereafter slightly shook the finally prepared solution.

Table 3.4: Formulation of blank hand sanitizer (without plant extract)

Elements	Value (ml)	Sources
IPA (75%)	80	WHO
Distilled Water	10	R&D
Glycerin (98%)	2	R&D

Honey	1.5	R&D
Olive oil	3	R&D
Hydrogen Per Oxide (3%)	1.5	WHO
Lemon extract	2	R&D

3.8.4 Preparation of nanoparticles hand sanitizer

In nanoparticles sanitizers instead of plant extract two types of nanoparticles solutions were used. At first two conical flask were taken. Then 80 ml of 75% Isopropyl alcohol (IPA) were taken each of the two conical flasks. Thereafter 1.5 ml honey, 3 ml olive oil and 2 ml of 98% glycerin were mixed separately with IPA in two conical flasks. After doing that stirred the solutions with a glass rod. Finally, 2ml plant extract of each nanoparticle's solution, 1·5 ml of 3% Hydrogen per oxide, 2ml lemon extract and 8 ml distilled water were mixed with previous solutions respectively. Then slightly shook the finally prepared solutions.

Table 3.5: Formulation of nanoparticles hand sanitizer:

Elements	Value (ml)	Sources
IPA (75%)	80	WHO
Distilled Water	8	R&D
Glycerin (98%)	2	R&D
Honey	1·5	R&D
Olive oil	3	R&D
Nanoparticles solutions (Eucalyptus and Neem)	2	R&D
Hydrogen Per Oxide (3%)	1·5	WHO
Lemon extract	2	R&D

3.9 Physical parameter evaluation

Physical appearance/visual inspection

In the food chemistry lab, the physicochemical properties of created hand sanitizers were determined. The color and appearance of the hand sanitizers were chosen based on their appearance. A sanitizing odor was detected.

Determination of pH

A digital pH meter was used to determine the pH values of all produced formulations. At room temperature (25°C), the measurement was performed in a pH meter that had previously been calibrated.

3.10 Collection of E. coli, Salmonella spp. and Staphylococcus aureus

E. coli and *salmonella* spp. were collected from commercial poultry liver. At first, 1gm of liver was mix with 9 ml of peptone water. Then incubate it for 24 hours at 37°C. After that MacConkey and EMB agar were used for the isolation and identification of E. coli and salmonella. By inoculating loop streaking was done in both agars. Large pink color in MacConkey and Green Metallic sheen in EMB were indicates the present of *E. coli*. For *salmonella*, incubate bacterial peptone water was grown in the XLD agar and black pink color indicates the presence of *salmonella spp*. Commercial *Staphylococcus aureus* was used in this test. The reference number is “ATTC *Staphylococcus aureus* 29213”.

3.11 Preparation of bacterial stock

For preparation and preservation of stock, 2-3 colonies of bacteria were taken in a freshly prepared Brain heart infusion (BHI) broth. Then incubate overnight at 37°C overnight for bacterial growth. After that 700 μL was taken from overnight culture + 300 μL of 50% glycerol was mixed in Eppendorf tube. Finally stored the Eppendorf tube at -40 to -80°C for long time preservation.

3.12 Preparation of stock solutions for hand sanitizer

2 ml of plant extract (contain 200 mg crude extract) of nine herbs such as as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Lata, Guava was dissolved in 2 ml Glycerin of 98%, 1·5 ml Honey, 3ml olive oil,80 ml of 75% IPA (iso propyl alcohol), 1·5 ml Hydrogen peroxide, 2 ml lemon extract.

3.13 Preparation of Dried Filter paper discs

Using a puncher, each disc was cut from No. 1 filter paper with a diameter of around 6 mm. The sterilized filter paper disc was then impregnated with 60 µl of experimental plant extract sanitizer after being autoclaved at 121°C for 15 minutes. Each disc was extracted from the extract using sterile forceps and placed in a sterilized Petri dish before being dried and applied to the agar surface on a plate.

3.14 McFarland Standard preparation

To make the 0.5Mcfardland turbidity standard, 1 percent sulfuric acid (H_2SO_4) and 1 percent barium chloride (BaCl2) were mixed together to produce a solution with precise optical densities. To make a 0.5Mcfardland standard, combine 0.05 mL of 1% $BaCl_2$ solution with 9.95 mL of 1% H_2SO_4 solution and mix to maintain a suspension. A 0.5McFardland turbidity standard has an optical density of 1.5x108 colony forming units per milliliter (CFU/ml), which is equivalent to the density of a bacterial solution. Visually compare the turbidity of the test suspension to that of the McFarland standard by comparing the clarity of the lines on the Wickerham card in the presence of excellent illumination. If the test suspension is too light, add more organisms or incubate the tube until it reaches the same turbidity as the standard. Use a sterile pipette to dilute the sample with enough broth or saline to achieve the same turbidity as the reference (McFarland, 1907).

3.15 Preparation of Agar media

Mueller-Hinton agar for *E. coli* and *Salmonella* spp.

1000 mL Mueller-Hinton agar (MHA) weighted amounts of 10 g tryptone, 15 g nutritional agar, 5 g sodium chloride, and 5 g yeast extract were added to the conical flask. 1000mL distilled water was mixed in. Boiling for 10 minutes to thoroughly dissolve all materials, then autoclaving for 15 minutes to sterilize and use for subculturing of the test microorganisms.

Blood agar for *Staphylococcus aureus*

To make 1000 mL of blood agar, follow these instructions. In a conical flask, 10 g of casein, 15 g of nutritional agar, 5 g of sodium chloride, and 2 g of yeast extract were weighted and added. 1000 mL distilled water was added to the mixture and stirred well. Boiling for 10 minutes to thoroughly dissolve all components, then autoclaving for 15 minutes to sterilize. When the temperature drops to 45-50°C, add 50 mL cow blood and stir thoroughly. The test bacteria were then sub-cultured on agar.

Figure 3.4: Preparation of Blood agar

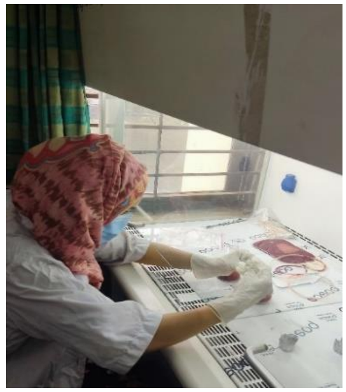

Figure 3.5: Add Blood to the solution

Figure 3.6: Streaking on the Agar media

3.16 Antimicrobial Activity test of plant hand sanitizer by Agar Disc Diffusion Assay

The disc diffusion technique was used to examine the antibacterial activity of the experimental plant's hand sanitizer against E. coli and salmonella spp. The Sterile Muller Hinton agar was prepared as follows for each organism. Twenty milliliters of sterile Muller Hinton agar (MHA) placed into sterilized Petri dishes (kept at 45-50°C in a molten condition). 0.1ml of test organism was put on MHA plate after Muller Hinton agar had solidified. The filter paper discs were then put on the agar plate's surface at identical distances from one another and 15 mm from the plate's edge, as specified by (Clutter buck *et al.*, 2007). Each disc was pushed down to establish complete contact with the agar surface, then inverted and put in a 37°C incubator for 24 hours. After 24 hours, the antimicrobial susceptibility was assessed by measuring the zone of inhibition with a plastic ruler in mm and comparing the results to the standard medication ampicillin as a reference standard and sterile saline discs as a negative control.

Figure 3.7: preparation of Muller Hinton agar

Figure 3.8: Handling the Autoclave

Figure 3.9: Antimicrobial activity test

3.17 Statistical analysis

The data of microbial tests were performed in triplicate and results are expressed as mean ± standard deviation (SD). The mean and standard deviation values were analyzed using SPSS 22 version.

Chapter 4: Results

This experiment was conducted to evaluate the physicochemical characteristics of the formualated hand sanitizers, market brand sanitizer, and Hexisol. In this study we also evaluate presence phytochemicals constituent of nine herbs (Tulsi, Neem, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Gulancho lata) and antimicrobial activity of formulated hand sanitizers, market brand hand sanitizer and Hexisol against three types of bacteria such as *E.coli Salmonella* spp. and *staphylococcus aureus*.

4.1 Phytochemicals screening

Here, eight phytochemicals such as Alkaloids, Flavonoids, Saponins, Tannins, Phenolic compound, Glycosides, Carbohydrates, Acidic compound are observed in the plant extracts of nine plants such as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata. The color change of plant extract which were defined in previous phytochemical screening method indicates the presence of phytochemicals. The study revealed that all eight phytochemicals are identified in Eucalyptus and Mahogany. It was invented that four phytochemicals were found in Lata, five phytochemicals were identified in Neem, seven phytochemicals are found in Tulsi, Ash gourd, Meadow grass, Tamarind, and six phytochemicals were identified in Guava. The presence of alkaloids has been detected in all plant extracts except Neem and Lata. Flavonoids and tannins have been found in all plant extracts. Moreover, in Tulsi, Eucalyptus, Mahogany, and Guava plant extracts saponins was present. In addition, glycosides is detected in all other plant extracts, not just guava. Again, Phenolic compound and acidic compound both are present in all plant extracts except Lata.

Table 4. 1: Phytochemical screening results

Test name	Neem	Tulsi	Eucalyptus	Mahogany	Ash gourd	Meadow Grass	Tamarind	Guava	Lata
Alkaloids	-	+	+	+	+	+	+	+	-
Flavonoids	+	+	+	+	+	+	+	+	+
Saponins	-	+	+	+	-	-	-	+	-
Tannins	+	+	+	+	+	+	+	+	+
Phenolic compound	+	+	+	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+	+	-	+
Carbohydrates	-	-	+	+	+	+	+	-	+
Acidic compound	+	+	+	+	+	+	+	+	-

4.2 Physical parameters of Hand sanitizers

The produced hand sanitizers' physicochemical characteristics were determined. Color, odor, appearance, and pH were all tested. The formulations have a pleasing look, and the sanitizer's overall characterization revealed that the formulations do not have a disagreeable color or odor. The pH of each plant hand sanitizer is mild acidic. The range of pH of the sanitizers are found in the range of 5·7 to 6·9. The pH of the sanitizers is given in the Ascending order: 6·9 (Tulsi) > 6·8 (Guava) > 6·7 (Blank) > 6·6 (Sepnil) > 6·5 (Ash gourd) >6·4 (Mahogany) > 6·3 (Neem) > 6·2 (Tamarind) > 6·1 (Mix herbs) > 5·9 (Eucalyptus) > 5·8 (Eucalyptus nanoparticles) > 5·7 (Lata). Here, the pH of the Tulsi sanitizer is highest and the pH of Lata is lowest.

Table 4.2: Physical parameter of hand sanitizers

Herbal sanitizer	Color	Aroma		Appearance	pH
		Before adding aroma	After adding aroma		
Neem	Brown	Strong garlic like	Lemon fragrance	Liquid	6·3
Tulsi	Dark green	Clove like	Lemon fragrance	Liquid	6·9
Eucalyptus	Dark Brown	Camphor like	Lemon fragrance	Liquid	5·9
Mahogany	Dark Brown	Sweet aroma	Lemon fragrance	Liquid	6·4
Ash gourd	Brownish	Not unpleasant	Lemon fragrance	Liquid	6·5
Meadow Grass	Yellow	Grass like	Lemon fragrance	Liquid	6·3
Tamarind	Dark Brown	Sweet	Lemon fragrance	Liquid	6·2
Guava	Dark Brown	Like Guava fruits	Lemon fragrance	Liquid	6·8
Lata	Green	Leaves like	Lemon fragrance	Liquid	5·7
Mix herbs	Dark Brown	Sweet	Lemon fragrance	Liquid	6·1
Neem Nanoparticle	Brown	Not unpleasant	Lemon fragrance	Liquid	6·2
Eucalyptus Nanoparticle	Dark Brown	Sweet	Lemon fragrance	Liquid	5·8
Blank	colorless	Alcoholic	Lemon fragrance	Liquid	6·7
Sepnil	colorless	Lemon like		Liquid	6·6
Hexisol	Blue	Strong		Liquid	5·9

4.3 Antimicrobial efficacy tests of fifteen types hand sanitizers

4.3.1 Antimicrobial efficacy of herbal sanitizers against *E.coli*

In this study antimicrobial efficacy tests were evaluated on nine herbal sanitizers and mix herbs sanitizer. The zones of inhibition of herbal sanitizers including Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata, and Mix herbs, on *E.coli* are 14·16 ± 1·04, 12·83 ± 0·76, 27·5 ± 0·50, 12·83 ± 1·04, 15·5 ± 1·32, 17·16 ± 1·25, 14·16 ± 0·76, 31 ± 1·00, 15 ± 0·50, 17·33 ±1·04 mm respectively. The control used on *E.coli* is Ciprofloxacin and the zone of inhibition of ciprofloxacin is 31·16 ±1·25mm.

4.3.2 Antimicrobial efficacy of herbal sanitizers against *Salmonella* spp.

According to antimicrobial efficacy against *Salmonella*, the zones of inhibition of herbal sanitizers including Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata, and Mix herbs are 21·83 ± 1·25, 20 ± 2·00, 22·83 ± 1·60, 21·33 ± 0·76, 20·33 ± 1·52, 20·16 ± 2·25, 18·83 ± 1·25, 25 ± 2·00, 20·16 ± 1·25, 26·33 ± 0·76, mm respectively. Enrofloxacin is used as control against *Salmonella* spp. and the zone of inhibition of control is 26·83± 0·76mm.

4.3.3 Antimicrobial efficacy of herbal sanitizers against *Staphylococcus aureus*

The zones of inhibition of herbal hand sanitizers such as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata, mix herbs, Neem nanoparticle, Eucalyptus nanoparticle, Blank, Sepnil, Hexisol against *Staphylococcus aureus* are 10·16 ± 1·25, 10 ± 0·5, 16 ± 0·5, 13·33 ± 1·25, 10·83 ± 0·76, 7·16 ±1·04, 16·5 ± 0·5, 11·33 ± 1·04, 8·5 ±0·5, 19·16 ± 1·04, mm respectively. The control used against *Staphylococcus aureus* is Ciprofloxacin and zone of inhibition is 24·16 ± 0·76 mm.

4.3.4 Antimicrobial efficacy of nanoparticles sanitizers

The zones of inhibition of Neem nanoparticles sanitizer and Eucalyptus nanoparticles sanitizer against *E.coli* are 19·5 ±1·32, 25± 2·00 mm respectively. Moreover, according to antimicrobial efficacy test against *Salmonella* spp., the zones of inhibition of Neem nanoparticles sanitizer and Eucalyptus nanoparticles sanitizer are 23·5 ± 1·32, 27·16 ± 1·04 mm respectively. In accordance with antimicrobial efficacy test the zones of inhibition of Neem nanoparticles sanitizer and Eucalyptus nanoparticles sanitizer on *Staphylococcus aureus* are 14·83 ± 0·76, 17·16 ± 1·25 mm respectively

4.3.5 Antimicrobial efficacy of comparing sanitizers

The zones of inhibition of Blank sanitizers against *E.coli, salmonella* spp. and *Staphylococcus aureus* are 8·83 ± 0·76, 9·5 ± 0·5, 5·5 ± 0·5 mm respectively. According to microbial efficacy against *E.coli, salmonella* spp. and *Staphylococcus aureus* the zones of inhibition of Sepnil (market brand sanitizer) were 21·16 ± 1·25, 20·5 ± 0·5, 12·16 ± 0·76 mm respectively which are smaller than the previously mentioned herbal hand sanitizers. Again, the zone of inhibition of Hexisol *E.coli, Salmonella* spp. *and Staphylococcus aureus* were 34·33 ± 1·52, 38·5 ± 1·32, 28·5 ± 0·5 mm respectively which were greater than previously mentioned herbal hand sanitizers.

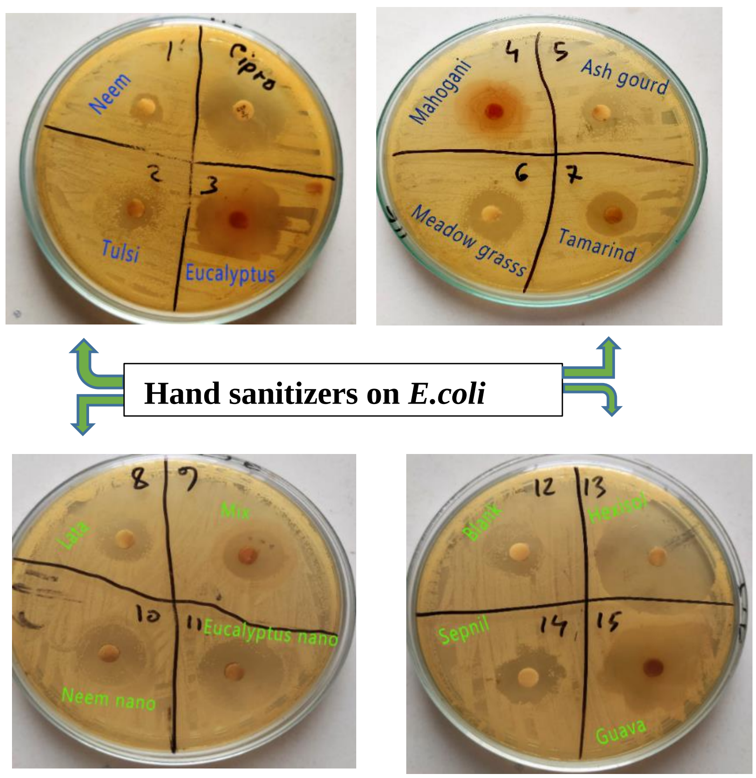

Figure 4. 1: Zone of inhibition of Hand sanitizers and ciprofloxacin on E.coli

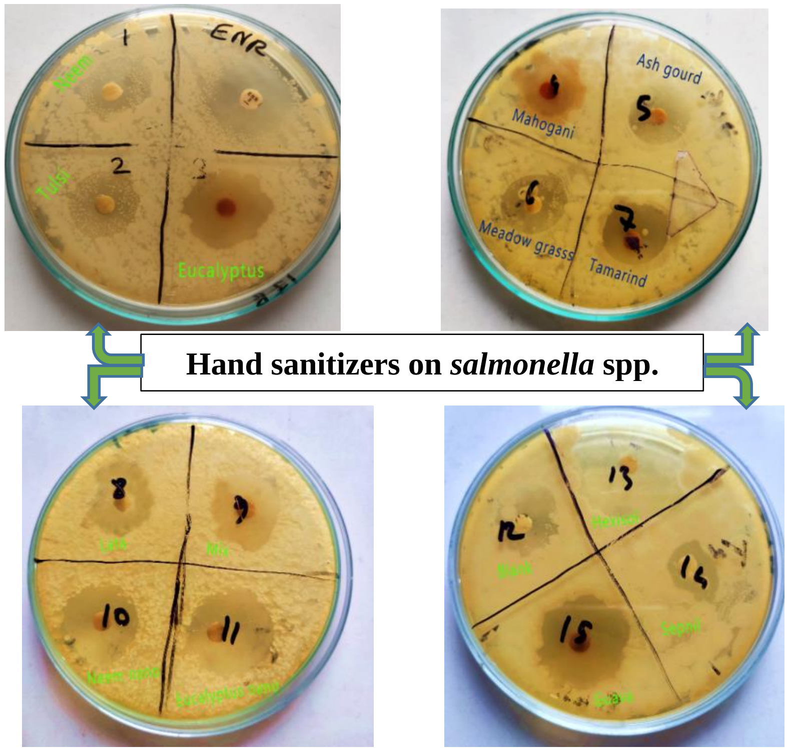

Figure 4. 2: **Zone of inhibition of Hand sanitizers and Enrofloxacin on *Salmonella* spp**

Figure 4. 3: Zone of inhibition of Hand sanitizers and ciprofloxacin on S.aureus

Table 4. 1: Zone of inhibition of herbal hand sanitizers against three types of bacteria such as *E. coli, Salmonella* spp. and *S. aureus*

Sample name(n)	*E.coli* Mean ± SD	*Salmonella* spp. Mean ±SD	*S.aureus* Mean ± SD
Neem(3)	14·16 ± 1·04	21·83 ± 1·25	10·16 ± 1·25
Tulsi(3)	12·83 ± 0·76	20 ± 2·00	10 ± 0·5
Eucalyptus(3)	27·5 ± 0·50	22·83 ± 1·60	16 ± 0·5
Mahogany(3)	12·83 ± 1·04	21·33 ± 0·76	13·33 ± 1·25
Ash gourd(3)	15·5 ± 1·32	20·33 ± 1·52	10·83 ± 0·76
Meadow grass(3)	17·16 ± 1·25	20·16 ± 2·25	7·16 ±1·04
Tamarind(3)	14·16 ± 0·76	18·83 ± 1·25	16·5 ± 0·5
Lata(3)	15 ± 0·50	20·16 ± 1·25	8·5 ±0·5
Mix herbs(3)	17·33 ±1·04	26·33 ± 0·76	19·16 ± 1·04
Neem Nanoparticle(3)	19·5 ±1·32	23·5 ± 1·32	14·83 ± 0·76
Eucalyptus Nanoparticle(3)	25± 2·00	27·16 ± 1·04	17·16 ± 1·25
Blank(3)	8·83 ± 0·76	9·5 ± 0·5	5·5 ± 0·5
Hexisol(3)	34·33 ± 1·52	38·5 ± 1·32	28·5 ± 0·5
Sepnil(3)	21·16 ± 1·25	20·5 ± 0·5	12·16 ± 0·76
Guava(3)	31 ± 1·00	25 ± 2·00	11·33 ± 1·04
Control	31	26	25

4.3.6 The leading sanitizers according to result on *E.coli*

When all sanitizers are ordered in an Ascending order, we found the leading sanitizers according to microbial efficacy against *E.coli*. The microbial efficacy of tested sanitizers against *E.coli* are given in the Ascending order: 34·33 ± 1·52 (Hexisol) > 31 ± 1·00 (Guava) > 27·5 ± 0·50 (Eucalyptus) > 25± 2·00 (Eucalyptus nanoparticles) >19·5 ±1·32 (Neem nanoparticles) > 21·16 ± 1·25 (Sepnil) > 17·33 ±1·04 (Mix herbs) > 17·16 ± 1·25 (Meadow grass) > 15·5 ± 1·32 (Ash gourd) > 15 ± 0·50 (Lata) > 14·16 ± 1·04 (Neem) >14·16 ± 0·76 (Tamarind) > 12·83 ± 1·04 (Mahogany) > 12·83 ± 0·76 (Tulsi) >8·83 ± 0·76 (Blank). So, Guava leaves hand sanitizer is the largest, Eucalyptus hand sanitizer is the second largest, Eucalyptus nanoparticle hand sanitizer is the third largest.

4.3.7 The leading sanitizers according to result on *salmonella* spp.

Here all sanitizers are ordered in an Ascending order, then we found the leading sanitizers according to microbial efficacy against *salmonella*. The microbial efficacy of tested sanitizers against *salmonella* spp. are given in the Ascending order: 38·5 ± 1·32 (Hexisol) > 27·16 ± 1·04 (Eucalyptus nanoparticles) > 26·33 ± 0·76 (Mix herbs) > 25 ± 2·00 (Guava) > 23·5 ± 1·32 (Neem nanoparticles) > 22·83 ± 1·60 (Eucalyptus) > 21·83 ± 1·25 (Neem) > 21·33 ± 0·76(Mahogany) > 20·33 ± 1·52 (Ash gourd) > 20·16 ± 2·25 (Meadow grass) > 20·16 ± 1·25 (Lata) > 20·5 ± 0·5 (Sepnil) > 20 ± 2·00 (Tulsi) > 18·83 ± 1·25 (Tamarind) > 9·5 ± 0·5 (Blank). So, Eucalyptus nanoparticle sanitizer is the greatest, mix herbs hand sanitizer is the second, Guava leaves hand sanitizer is the third.

4.3.8 The leading sanitizers according to result on *Staphylococcus aureus*

After ordering in an Ascending order, we found the leading sanitizers. The microbial efficacy of tested sanitizers against *Staphylococcus aureus* are given in the Ascending order: 28·5 ± 0·5 (Hexisol) > 19·16 ± 1·04 (Mix herbs) > 17·16 ± 1·25 (Eucalyptus nanoparticles) 16·5 ± 0·5 (Tamarind) 16 ± 0·5 (Eucalyptus) 14·83 ± 0·76 (Neem nanoparticles) 13·33 ± 1·25 (Mahogany) 12·16 ± 0·76 (Sepnil) 11·33 ± 1·04 (Guava) 10·83 ± 0·76 (Ash gourd) 10·16 ± 1·25 (Neem) 10 ± 0·5(Tulsi) 8·5 ±0·5 (Lata) 7·16 ±1·04 (Meadow grass) 5·5 ± 0·5 (Blank). So, mix herbs hand sanitizer has a wider zone of inhibition on *Staphylococcus aureus* Eucalyptus nanoparticle hand sanitizer is second and tamarind leaves hand sanitizers is third than others.

Chapter 5: Discussion

The study investigated that the formulation of herbal sanitizers as well as nanoparticle sanitizers. Silver nanoparticles solution which acts as antimicrobial agent was used in nanoparticles hand sanitizers. In addition, the study also involved in identifying phytochemicals which has antimicrobial effect. Moreover, the formulated sanitizers were compared with blank sanitizers, Market brand sanitizer (Sepnil), and Hexisol according to antimicrobial efficacy test against three types of bacteria including *E.coli*, *Salmonella* spp., and *Staphylococcus aureus*

5.1 The presence of phytochemicals in plant extracts

Plants and portions of the same plant have varied phytochemical contents. The medicinal activities of plants are attributed to alkaloids, flavonoids, saponins, terpenoids, steroids, phlobatannins, glycosides, tannins, and other chemicals. All of these secondary metabolites have been linked to the treatment of various diseases. Antispasmodic, antimalarial, analgesic, and diuretic properties are only a few of the properties of alkaloids. Saponins have anti-inflammatory, antiviral, and antifungal properties, as well as the ability to decrease cholesterol. Antifungal and antibacterial properties of glycosides have been demonstrated. Antioxidant, anti-allergic, antimicrobial, and other properties have been discovered in phenols and flavonoids (Soni and Sosa, 2013).

Vijaya et al., 2021 investigated on preliminary phytochemicals such as phenolic compound, terpenoid, triterpenes, tannin, saponins, steroids, flavonoids screening of *Azadirachta indica* and phytochemicals including alkaloids, flavonoids, saponins, terpenoids, glycosides, sterols screening of *Eucalyptus globulus*. They found that three phytochemicals such as Phenolic compound, steroids, and flavonoids were found in methanolic extract of *Azadirachta indica* (Neem) and five phytochemicals such as Tannin. Terpenoids, flavonoids, saponins, sterols were identified in methanolic extract of *Eucalyptus globulus* (Eucalyptus). They did not find terpenoid, triterpenes, tannin, saponins in the methanolic extract of *Azadirachta indica*. Moreover, they did not identify alkaloids and glycosides in the methanolic extract of *Eucalyptus globulus*.

On the other hand, we investigated on preliminary screening of eight phytochemicals including Alkaloids, Flavonoids, Saponins, Tannins, Phenolic compound, Glycosides, Carbohydrates, Acidic compound of nine methanolic plant extracts such as Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata. The results revealed that five phytochemicals such as Flavonoids, Saponins, Tannins, Phenolic compound, Glycosides were identified in the methanolic extract of Neem. In addition, previously mentioned eight phytochemicals tested were identified in the methanolic extract of Eucalyptus.

5.2 The pH of hand sanitizers

The pH of designed hand sanitizers was found to be in the moderate acidic range of 5·7 to 6·9. Hand sanitizer pH has been demonstrated to be crucial for improving and enhancing hand quality, decreasing hand irritation, and regulating the skin's ecological balance. The recent trend to flourish hand sanitizer of mild pH is one of the ways to reduce skin damages. Mild acidity prevents edema and flourishes scale tightness, there by inducing shine. (Nandkishor, 2013).

5.3 The antimicrobial efficacy of herbal hand sanitizers

In this study we investigated on antimicrobial efficacy of fifteen hand sanitizers including Neem, Tulsi, Eucalyptus, Mahogany, Ash gourd, Meadow grass, Tamarind, Guava, Lata, Mix herbs, Sepnil, Hexisol against three types of bacteria such as *E.coli*, *Salmonella* spp. and *Staphylococcus aureus*. Among the sanitizers the zone of inhibition of Guava leaves hand sanitizer was the largest (31 ± 1·00 mm**)**, Eucalyptus hand sanitizer was the second largest (27·5 ± 0·50 mm**)**, and Eucalyptus nanoparticle hand sanitizer was the third largest (25 ± 2·00 mm) against Escherichia *coli*. Furthermore, among sanitizers the zone of inhibition of Eucalyptus nanoparticles sanitizer was greater (27·16 ± 1·04 mm**),** mix herbs hand sanitizer was second (26·33 ± 0·76 mm**)** and Guava leaves hand sanitizer was third (25 ± 2·00 mm**)** than others on *Salmonella* spp. In addition, among the sanitizers mix herbs hand sanitizer had a wider zone of inhibition on *Staphylococcus aureus* (19·16 ± 1·04 mm**)**, Eucalyptus nanoparticle hand sanitizer was second (17·16 ± 1·25 mm**)**, and tamarind leaves hand sanitizers was third (16·5 ± 0·5 mm) than other hand sanitizers. Here, Guava leaves hand sanitizer is the best in accordance with antimicrobial efficacy against *E.coli*, Eucalyptus nanoparticles sanitizer is the greatest according to antimicrobial efficacy on *salmonella* spp. and Mix herbs sanitizers is the best in accordance with *Staphylococcus aureus*. Therefore, these three sanitizers are the leading sanitizers.

A study from Jogu Chandrudu, 2019 we found that the zones of inhibition of polyherbal sanitizer prepared by Neem extract, Coriander extract, Aloe Vera gel, Lemon oil, alcohol, glycerin, sodium laurate sulfate (30%), rose oil, annatto seeds against *E.coli* and *Staphylococcus aureus* are 28 ± 0·2 and 23 ± 0·12 respectively. Again, they found the zone of inhibition of polyherbal sanitizer prepared by previously mentioned all ingredients except coriander extract against *E.coli* and *Staphylococcus aureus* are 23 ± 0·28 and 22 ± 0·02 respectively. Here we are got on to that the zone of inhibition of our leading sanitizer (Guava leaves sanitizer) is greater than zone of inhibition of their formulation against *E.coli*. Again, the zone of inhibition of our leading sanitizer (Mix herbs sanitizer) is smaller than their formulation against *Staphylococcus aureus*.

In one investigation Jyotsana et al., 2021 showed antimicrobial activity in polyherbal sanitizer prepared by isopropyl alcohol, hydrogen peroxide (H_2O_2), ethanol, glycerol, camphor, mixture of all plant extracts such as *Ocimum gratissum* (Van tulsi), *Ocimum sanctum* (Shyama tulsi), *Eucalyptus globules* (Niligiri) and *Azadiracta indica* (Neem), *Cuscuta reflexa* (Amarbel), *Aloe barbadensis (*Ghritkumari) and *Menthe arvensis* (Mint) against the strains used in *S. aureus* and *E. coli* and gave zone of inhibition of 11 ± 0.01 and 7 ± 0.001 mm respectively. From the above study, we found that the zone of inhibitions of our leading sanitizers against *Escherichia coli* are greater than the polyherbal sanitizer of Jyotsana et al., 2021. Moreover, the zone of inhibitions of our leading sanitizers against *Staphylococcus aureus* are wider than polyherbal sanitizer of Jyotsana et al., 2021. So, it is understood that the zone of inhibitions of the leading sanitizers made by us are bigger than the sanitizers made by them.

5.4 Antimicrobial efficacy of silver nanoparticles-based sanitizers

In present study two types of nanoparticles solutions were used to formulate nanoparticles sanitizers. Among the nine plant extracts only two plant extracts including Neem extracts and Eucalyptus extracts formed nanoparticles in the presence of silver nitrate solution. The antimicrobial efficacy of nanoparticles sanitizers was amazing. According to previous results we found that microbial efficacy against *E.coli* the zone of inhibition of Eucalyptus nanoparticles was 25 ± 2·00 mm which was third largest sanitizers among formulated sanitizers. The zone of inhibition of Eucalyptus nanoparticles sanitizer Against *Salmonella* was 27·16 ± 1·04 mm which indicated that it was one of the leading sanitizers. The antimicrobial efficacy test against *Staphylococcus aureus* revealed that the zone of inhibition of Eucalyptus nanoparticle hand sanitizer was 17·16 ± 1·25 mm which was the second largest. The antimicrobial efficacy of Neem nanoparticles is moderate good. The zones of inhibition against *E.coli*, *Salmonella* spp. and *S.Aureus* are 19·5 ±1·32, 23·5 ± 1·32, 14·83 ± 0·76 mm respectively. So, nanoparticles sanitizers are impressive to kill microorganisms.

Several hypotheses concerning the antibacterial characteristics of silver nanoparticles have been identified. Silver nanoparticles have been shown to be capable of inactivating bacterial enzymes by releasing ionic silver, which inactivates the thiol groups. Silver ions interfere with bacterial DNA replication, damage cell cytoplasm, reduce adenosine triphosphate (ATP) levels, and eventually kill cells. (Bhattacharya and Mukherjee, 2008). Silver nanoparticles have been found to have superior antimicrobial activity against bacteria, fungus, viruses, and other eukaryotic microorganisms. (Savithramma et al., 2012).

5.5 Comparison of the herbal sanitizers with market brand sanitizer and Hexisol

Among the sanitizers the zones of inhibition of sepnil on *E.coli, Salmonella* spp. and *Staphylococcus aureus* are 21·16 ± 1·25, 20·5 ± 0·5, 12·16 ± 0·76 mm respectively. Furthermore, the zones of inhibition of Hexisol on *E.coli, Salmonella* spp. and *Staphylococcus aureus* are 34·33 ± 1·52, 38·5 ± 1·32, 28·5 ± 0·5 mm respectively. So, the zones of inhibition of sepnil against the mentioned bacteria are smaller than the leading sanitizers. Again, the zones of inhibition of Hexisol are larger than the leading sanitizers. But both sepnil and Hexisol contain synthetic products which may cause irritation, dryness, itchiness and allergens.

A study shows that irritating contact dermatitis has been linked to quaternary ammonium compounds, iodine or iodophors, chlorhexidine, triclosan, chloroxylenol, and alcohols, whereas allergic contact dermatitis has been linked to quaternary ammonium compounds, iodine or iodophors, chlorhexidine, triclosan, chloroxylenol, and alcohols. (Khokhar et al., 2020).

So, the herbal hand sanitizers that mentioned here are the claimants of good quality hand sanitizers considering all aspects.

Major Strengths of the study

- Formulation of twelve different alcohol based herbal sanitizers which are not found in other studies.
- Formulation of two types of nanoparticles sanitizers.
- Assessment of antimicrobial efficacy of the twelve types of herbal sanitizers against three types of bacteria.
- Comparing the formulated sanitizers with market brand sanitizers.

Major Limitations of the study

- Microbial tests were performed on only three types of bacteria.
- Only eight types of phytochemicals have been tested.
- No tests were performed on the virus and fungal growth.
- Some parameters such as viscosity, homogeneity, dirt dispersion ability, stability and solid content were not observed.
- Only three Physical parameters such as color, odor, and pH were evaluated
- The pH of Hand sanitizers was not observed over time.

Chapter 6: Conclusion

The study concludes that potential findings were used to manufacture twelve varieties of hand sanitizers from extracts of nine plants that were proven to exhibit antibacterial activity and effectiveness when compared to a commercially available reference hand sanitizer. Furthermore, phytochemical screening tests were performed to determine the presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins, phenolic compound, glycosides, carbohydrate, and acidic compound in the extracts of plants. All eight mentioned phytochemicals were found in the Eucalyptus and Mahogany plant extracts. In contrast, some plant extracts contained fewer than eight phytochemicals. The physicochemical assessment showed that the formulations have acceptable visual characteristics, and the overall characterization of the sanitizer revealed that the formulations have no unpleasant color or odor. The pH of all formulated herbal hand sanitizers was found in the range of 5·7 to 6·9. The study found that the zone of inhibition against *E.coli* of Guava leaves hand sanitizer is the largest (31 ± 1·00 mm**)**. In addition, the zone of inhibition on *Salmonella* spp. of Eucalyptus nanoparticle sanitizer is greater (27·16 ± 1·04 mm**)** than any other hand sanitizers. Furthermore, mix herbs hand sanitizer has the greatest zone of inhibition on *Staphylococcus aureus* (19·16 ± 1·04 mm**)**. According to results, we found that the zones of inhibition of these leading hand sanitizers were larger than sepnil. On the other hand, the zones of inhibition of these leading hand sanitizers were smaller than Hexisol. Though the microbial efficiency of Hexisol is better than previously mentioned sanitizers, the Hexisol contain synthetic chemical which may cause irritation and allergens. Above all, from the discussion we have come to the conclusion that the herbal hand sanitizers we have made are of better quality than other hand sanitizers.

Outcomes of the study

- Among nine plants eight types of phytochemicals are present in Eucalyptus and Mahogany. On the other hand, reminiscence possesses less than eight phytochemicals.
- According to Microbial test, the zone of Inhibition of Guava leaves hand sanitizer on *E.coli* is larger, Eucalyptus hand sanitizer is second larger and Eucalyptus nanoparticle hand sanitizer is third larger than any other hand sanitizer.

- In accordance with microbial test, the zone of inhibition on *Salmonella* spp. of Eucalyptus nanoparticle sanitizer is larger, mix herbs hand sanitizer is second larger and Guava leaves hand sanitizer is third larger than others.
- According to microbial test, the zone of inhibition on *Staphylococcus aureus* of mix herbs hand sanitizer is larger than other hand sanitizers and Eucalyptus nanoparticle hand sanitizer is second larger and Tamarind leaves hand sanitizer is third larger than other hand sanitizers.
- The results of physical parameter have shown that the pH of all herbal hand sanitizers was found in the range of 5·9 to 6·2 and the formulated sanitizers did not show any bad color and odor

Chapter 7: Recommendation

The formulated herbal hand sanitizers using nine plant extracts, glycerin, olive oil, hydrogen peroxide and isopropyl alcohol has shown drastic killing activity of the mentioned bacterial species. This test was performed on only three types of bacteria such as *E.coli*, *Salmonella* spp. and *Staphylococcus aureus*, so the effectiveness of the hand sanitizers will be better understood later on by testing on some more bacteria. The formulation must be evaluated against viruses and fungi that are often affected. Only eight types of phytochemical assays were performed in this study. So, some more phytochemical tests should be done to better understand the properties of plants. The plant extract used here is made from methanolic plant extract. In further research, hand sanitizers can be made from ethanolic plant extract and hand sanitizer of methanolic extract can be compared with it. It is also advised that further research be done on the toxicity of prepared hand sanitizers before they are used on people's hands. Another tip is to look into the severity of children accidentally ingesting hand sanitizer. Hand sanitizer is manufactured in liquid form in this study; therefore, it will be interesting to see how effective it will be if made in gel form. An attempt can be made to manufacture a water-based or non-alcohol-based herbal hand sanitizer and define it using the appropriate criteria. A comparison of the antibacterial activity of the previously designed alcohol-based herbal hand sanitizer and the newly formulated non-alcohol-based herbal hand sanitizer can be conducted to determine which is superior. Here, the pH of sanitizers was not tested overtime. So, it remains to be seen whether the pH changes after a period of 3 to 4 months. By doing so, the effectiveness of sanitizers will be better understood.

Chapter 8: References

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Brief biography of the author

This is Sayema Khanam Siddika, a candidate for the degree of MS in Food Chemistry and Quality Assurance, Department of Applied Chemistry and Chemical Technology, Faculty of Food Science and Technology, CVASU. She passed the secondary School Certificate Examination from Chittagong Govt. Girls' High School in 2011. Then she passed the Higher Secondary Certificate Examination from Kapashgola City Corporation Mohilla College in 2013. She obtained her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology of Chattogram Veterinary and Animal Sciences University, Chattogram. She has immense zeal in Food chemistry, Phytochemistry and new product development.

Appendices

Grinding process

Methanolic solution preparation

Filtering the Methanolic solution

Extraction by rotary evaporator

Determination of pH of the sanitizers

Research and Development process

Phytochemical test

Phenolic compound test

Alkaloids tests result



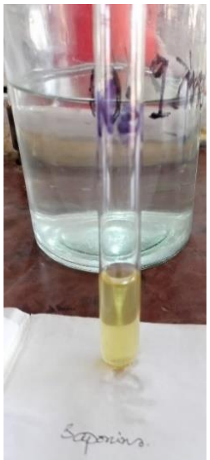



Tulsi **Eucalyptus** **Ash gourd** **Meadow grass** **Tamarind** **Guava**

Flavonoids test result

All herbs flavonoids test result

Saponin test results



Neem

Tulsi

Eucalyptus

Mahogany

Ash gourd

Meadow grass

Mahogany

Guava

Lata

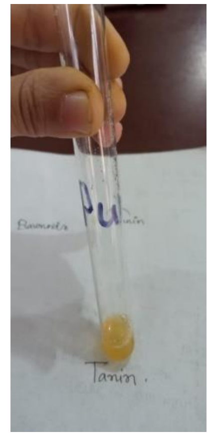
Tannin test results

Neem

Tulsi

Eucalyptus

Mahogany



Ash gourd

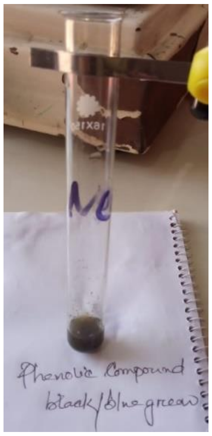
Mahogany

Meadow grass

Guava

Lata

Phenolic compound test result



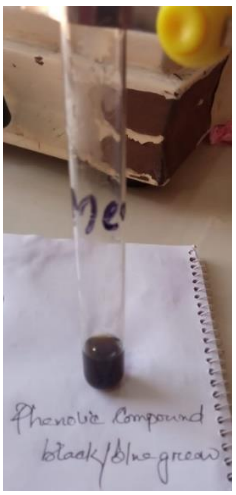
Neem

Tulsi

Eucalyptus

Mahogany

Ash gourd



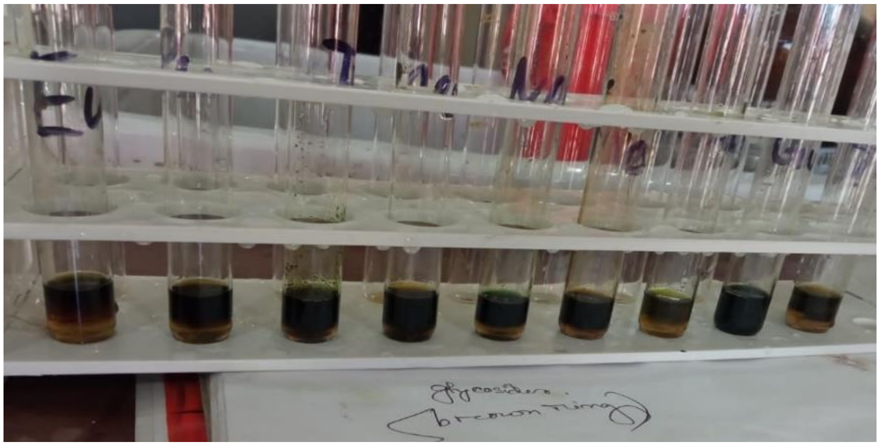
Meadow grass

Tamarind

Guava

Lata

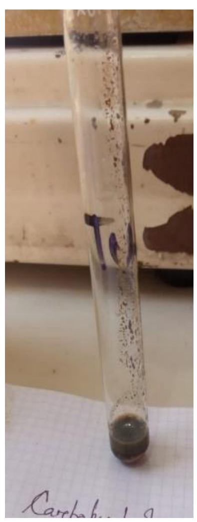
Glycosides test results



All herbs glycosides test result

Carbohydrate test results

Neem

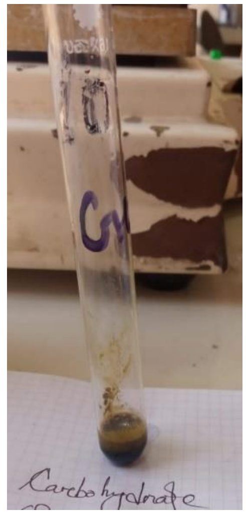


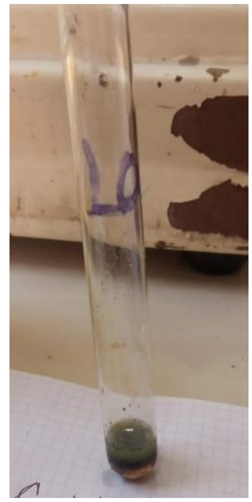
Tulsi

Eucalyptus

Mahogany

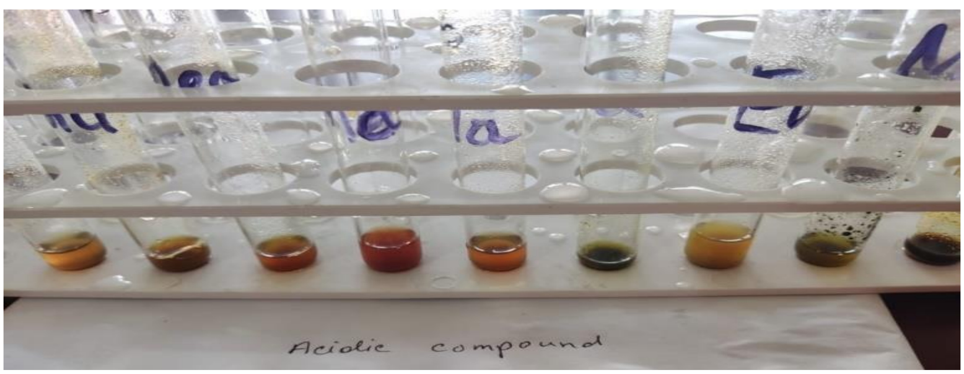
Ash gourd





Meadow grass **Tamarind** **Guava** **Lata**

Acidic compound test results



All herbs acidic compound test results