Formulation, Physicochemical and Antibacterial Activity of Polyherbal Hand Sanitizer

A Thesis Submitted to Office of Graduate Studies of Adama Science and Technology University in Partial Fulfillment of the Requirements for the Degree of Master of Science in Industrial Chemistry

By

Lelo deme

Adama, Ethiopia
June, 2018

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Approval sheet

We undersigned, members of the board of examiners of the final open defense by Lelo Deme have read and evaluated her thesis entitled with "Formulation, Physicochemical and Anti-bacterial Activities of Polyherbal Hand Sanitizer" and examined the candidate. This is therefore, to certify that the thesis has been accepted in partial fulfillment of the requirement of the degree of Master of Science in applied chemistry with specialization in Industrial chemistry

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DECLARATION

I, here declare that this thesis is my original work and has not been presented for degree or diploma in any other university. All source or materials used for this thesis have been duly acknowledged

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ACKNOWLEGMENTS

First and most importantly, I would like to acknowledge God for treasured gifts of well-being, power, trusts, love, hopefulness, tolerance and fortification to me and my families. Next, I would like to acknowledge Dr. Milkyas Endale (co-advisor) for his follow up throughout my work and Dr. Tegene Desalegn (main advisor) for his comments and valuable ideas. I would like to acknowledge all laboratory assistants of Applied Chemistry, Biology, Chemical and Material Science and Eng'g Departments for access to some instruments needed during the laboratory work and Regional laboratory or Oromia public health research capacity building and quality assurance laboratory, Adama,Ethiopia for bioassay determining.

The last but not the least, I would like to acknowledge Adama Science &Technology University, Department of Applied Chemistry for sponsoring MSc study.

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List of Abbreviations and Acronyms

Abbreviation	Symbol
Alcohol-based hand rubs	ABHR
British Columbia Centre for Disease Control	BCCDC
Eosin Methylene Blue agar	EMB
Food and Drug administration	FDA
Hand sanitizer	HS
Minimum bactericidal concentration	MBC
Minimum inhibitory concentration	MIC
Parachlorometaxylenol	PCMX
Triethanolamine	TEA
Tripticase soy broth medium	TSB

Abstract

Hand sanitizer disinfects harmful microorganisms on hand and avoids antagonistic belongings such as itching, annoyance, and dermatitis. The main objective of this paper is formulation of alcohol based and alcohol free polyherbal hand sanitizer. Various essential oils, water and ethanol extracts of Aloe barbaddenis leaf, Citrus aurantifolia dulsis (Lime) leaf, Mentha spictata (spearmint) leaf, Ginger and Ocimum Cufudontii leaf were components of the hand sanitizers instead of synthetic ingredients. Physico-chemical parameters such as pH, viscosity, and foam stability were determined along with antibacterial activity of hand sanitizer on selected strains of microorganisms. The result shows that the formulation exhibit good appearance characteristic as well as the general characterization of the sanitizer proved that the formulations do not show any unpleasant color and odor. The observed anti-bacterial activity results demonstrate that all formulated poly herbal hand sanitizer can inhibited the growth of one or more of the selected pathogenic bacterial species. It was revealed that hand sanitizer formulated with 5% plant extract (HS9 and HS10) showed promising antibacterial activity against Entrococcus feasilis, Staphylococcus aureus,Pseudomonas aeruginosa, Klebsiella pneumonia,E.coli and shigella sonnie. The highest activity was observed for HS9 against Staphylococcus aureus with zone of inhibition 23.33±2.08. when compared to the reference hand sanitizer taken from market that shows anti-bacterial activity against S.auereus only with its zone of inhibition 7±1,the present formulated polyherbal hand sanitizer was more effective which has activity on all the selected pathogens. Thus the observed antibacterial results suggest that effective hand sanitizer can be formulated with the help of plants extracts.

***Keywords**: Essential oil, hand sanitizer, hand hygiene, plant extracts*

1. Introduction

1.1 Background of study

Skin, especially hands needed to be protected from bacterial pathogens as they are the most exposed part of the body. Proper hand care has long been cited as an effective way of reducing the spread of germs, diseases, and other contaminants. Handwashing is the sole greatest effective and cost effective means for avoiding transmission of infectious disease by hand. According to the British Columbia Centre for Disease Control (BCCDC), eight percent of common infections are spread by hand, and washing hands five times a day could extremely decrease the frequency of influenza (flu) and nosocomial infections (BCCDC, 2014). Washing hands with water by adding certain ingredients is therefore the simple matter to eliminate and minimize germs on hands. Along with increasing people's activities, especially in urban areas, and many instant products, then comes to the innovative waterless hand cleaners known as hand sanitizer (Mercuy, 2009).

Typically, hand care has involved the washing of hands with soap followed by rinsing the hands with water to remove the soap. However, antimicrobial products that do not require a water rinse because they evaporate off hand. Such products are therefore known to be rinseless hand sanitizers. They have become popular in recent years as another way of sanitizing hands. When such a rinseless hand sanitizer is used, the user applies the product to his/her hands and rubs the hands together so that the product eventually either evaporates or is absorbed into the skin. These antimicrobial products come in the form of lotions, liquid, gels, and foams. Antimicrobial agents that may be used in such products include alcohols, trichlorohydroxy diphenyl ether (Triclosan), parachlorometaxylenol (PCMX), and quaternaryammonium compounds. Among all usable chemicals, ethanol, isopropanol and n-propanol (in the order of increasing efficacy) are the strongest and fasten agents. The most commonly used concentrations alcohol in hand sanitizer formulation ethanol (60–90%), isopropanol (70–80%) and n-propanol (60-70%) (Rotter, 1999).

It has been suggested that, the most important way to prevent the transmission of dangerous diseases is to frequently wash hands with soap and water. The options for keeping hand clean include antimicrobial solutions, iodine, alcohol solutions and rubs. Hand sanitizer is a liquid generally used to decrease infectious agents on the hands (Vyas, 2011).

The literature review for this study indicated that rinse-free hand sanitizers are, by definition, intended for degerming skin without the aid of rinsing with water. This type of product has steadily

gained popularity in professional circles as a supplement to hand washing with soap and water. The types of rinse-free hand sanitizers generally are grouped into two broad categories alcohol-based products and alcohol-free products (Kramer, 2002). These products vary greatly in composition, ranging from 54% iso-propanol to 70% ethanol. The choice of this type of rinse-free antimicrobial product often is subjective and mainly based on factors such as cost, Presence of emollients in the formula, fragrances, delivery vehicle (eg, gel, foam), size, and marketing. Selection is less often based on the product's effectiveness at eliminating bacteria after a single application.

Many of chemical antiseptics available in the market are alcohol based sanitizers. These formulations including soaps and solutions reduce health care associated transmission of contagious diseases but they have some short comings or adverse effects, their frequent use can lead to skin irritation and also resistance among pathogens (Jayant *et al*, 2015). Plants are rich in a wide variety of secondary metabolites, such as phenolic compounds, tannins, terpenoids, alkaloids, and flavonoids, which have been found to have antimicrobial properties. Hence there is an upsurge of developing herbal disinfectants and evaluate its efficacy (Cowan, 1999).

There are many possible advantages of using natural products as antimicrobial compounds, such as fewer adverse effects, better patient tolerance, relatively inexpensive, renewability and biodegradability. Bearing in mind this requirement; an endeavor has been made to display classical collected works for the herbs with antimicrobial properties to formulate low cost polyherbal hand sanitizer from *Citrus Aurantifolia Dulcis* (Lime) Oil, *Mentha spictata* (Spearmint) Leaf Oil, water and ethanol extract of *Aloe barbaddenis leaf*, *Ginger* and *Ocimum leaf* which are known to have antimicrobial activities against disease caused by microorganism. The chemical analysis of different parts of these plants has been reported. It was found to contain Flavonoids, phenolic compounds, and proanthocyanidins. Studies revealed that the extracts of root of *C. aurantifolia* have been found effective in inhibiting the growth of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Beta-haemolytic streptococci*, *Escherichia coli* and *Neisseria gonorrhoeae* (Aibinu *et al*, 2007).

Thus, the present work was attempted to formulate polyherbal hand sanitizer including combination of different concentration of plant extracts such as *Citrus Aurantifolia Dulcis* (Lime) juice, *Mentha spictata* (Spearmint) Leaf oil, water and ethanol extracts of *Aloe barbaddenis Ginger* and *Ocimum cofudontii* as they possess different medicinal and chemical properties useful for formulating herbal

hand sanitizer. After formulation, physico-chemical parameters and antimicrobial activity was evaluated and compared with the refrence hand sanitizer from market.

1.2 Statement of Problem

Human hands are the most common means of transportation to transmit health care associated organisms. Germs can get onto hands from items we touch during daily activities and make us sick. In developing country economically the using of hand sanitizer is very rare except in higher education place, health care and in some urban area in which some people use it.

In places where there is no the availabilities of soap and water that can clean hand, peoples simply ingesting food along with microorganisms and exposed to infectious diseases. Thus, there is a need to formulating a low cost polyherbal hand sanitizer that can be accessed by majority of citizens. Moreover, some commercial sanitizer contains synthetic disinfecting substances that may damage the skin. Thus, formulating a green hand sensitizer that contains plant extracted disinfectants is best alternative.

1.3 Significance of study

The significance of formulation of the present polyherbal hand sanitizer is that its use, which has antimicrobial activities to reduce germs on hand and for conditioning. Synthetic derivatives have been used as moisturizing agents and disinfecting agents in hand sensitizers. But in most case using synthetic based hand sanitizer irritates and dries hand. A more radical approach in reducing the synthetic ingredients is by incorporating natural extracts whose functionality is comparable with their synthetic ingredients. The main advantage of using natural source is that they are easily available, cheap and harmless compared to chemical products. In this study the herbal extract based formulations were formulated which used to reduce germs on hand and harmless to hand and alternative to synthetic drugs. This job is therefore expected to produce self-preserving polyherbal hand sensitizer by using essential oil extract of Citrus *aurantifolia Dulcis* (Lime) and *Mentha spictata* (Spearmint) Leaf and water and ethanol extract of *Aloe barbaddenis*, *Ginger* and *Ocimum cufudontii* leaf.

1.4 Objectives

1.4.1 General objectives

The overall objective of this project is to formulate polyherbal hand sanitizers from combination of essential oils of *Mentha spictata* (Spearmint) leaf, *Citrus aurantifolia* leaf and fruit, water and ethanol extract of Ginger, *Aloe barbaddenis leaf* and *Ocimum cufudontii* leaf.

1.4.2 Specific objectives

- To extract essential oil from leaves of *Mentha spictata* (Spearmint) and *Citrus aurantifolia Dulcis* (Lime) leaf
- To extract *Aloe barbaddenis, Ocimum* cufudontii leaves and *Ginger* using water and ethanol solvents
- To formulate polyherbal hand sanitizer enriched with different concentration of natural plant extracts for both alcohol and alcohol-free hand sanitizers
- To evaluate the physicochemical parameters such as pH, viscosity, dirty dispersion test, percent of solid content and physical appearance of the formulated hand sanitizer
- To evaluate antibacterial activities of the formulated polyherbal hand sanitizer against six selected bacterial strains such as (*Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC-22953), Entrococcus feasilis (*ATCC 229212*), P. aeroginosa (*ATCC 27853*), Klebsiella pneumonia (*ATCC 700603) and *Shigella sonnie* (ATCC 2593) and compare to that of reference hand sanitizer from market

2. Literature review

2.1 Hand sanitizer

Hygiene is defined as the maintenance of the practice of cleanliness which is of most importance in the maintenance of well-being. Keeping body hygiene and using the cleanser are necessary for a healthy life. These concepts underscore the need to maintain hygiene in disease prevention.Although good & simple hygiene technique is single most important, easy and least expensive means of preventing health care-associated (nosocomial) infections and the spread of antimicrobial multidrug resistance; but, unfortunately poor hand-hygiene practices are still observed due to lack of scientific knowledge, unawareness of risks and unavailability of hand- hygiene facilities (Vyas, 2011).

Skin hygiene, particularly of hands, is considered to be one of the primary mechanisms to reduce risk of transmission of infectious agents by both the contact and fecal- oral routes. But depending on the product used, washing can raise the pH of the skin. Long-term changes in skin pH can cause skin damage, increased skin shedding, since some of the antibacterial characteristics of the skin are associated with its normally acidic pH (approximately 6.0). With prolonged soap contact, skin pH can reach 7.0-8.5 and remain high for 3-4 h. Soaps and detergents, particularly those that are anionic or cationic, are the most damaging of all substances routinely applied to skin (Larson, 1999).

Typically hand sanitizers were grouped into alcohol based and non-alcohol based. Alcohol-based hand sanitizers exist in liquid, foam, and easy-flowing gel formulations. Although 62% alcohol based sanitizers were commonly used, most alcohol based hand sanitizers contain between 60% and 85% alcohol (Kramer, 2002). Although alcohol-based formulas that comply with federal composition standards generally are considered effective, alcohol-based antiseptic hand wash preparations are flammable and do not demonstrate persistent antimicrobial activity. Also, repeated use often can cause drying and irritation of the skin. Alcohol strips the skin of essential oils and sebum, which act as a natural protective barrier against bacterial infection and precipitate protein. When applied to wounds or raw surfaces, therefore, it not only increases the risk of injury, but also forms a coagulum under which bacteria may subsequently thrive. It is, therefore, not useful for the disinfection of open lesions or abraded, inflamed skin. Together, these and other adverse properties

greatly limit the alcohol-based antimicrobial product's immediate effectiveness and increase the chances for the spread of infection (Paulson, 1994).

In the past few decades, several studies were performed to indicate antibacterial effect of hand gel sanitizer in different settings such as extended care facilities, schools and hospitals (Center for Disease Control, 2008). However, previous studies showed that not all sanitizers are equally effective in eradicating all germs (Garner, 1986, Centers for Disease Control, 2003). While some studies reported high efficacy of hand sanitizers in reducing the microbial flora of hand, other studies failed to show such efficacy of hand sanitizers (Boyce *et al*, 2002, FDA, 1994, Blaney, 2011). However, with different types of sanitizers being used in different public premises, the public may not be aware of why one type is preferred over the other, specifically comparing alcohol-based hand rubs (ABHR) and alcohol free hand sanitizers. There are advantages of using either type of sanitizer but according to the Wall Street Journal, there is insufficient real world evidence to demonstrate that the alcohol free hand sanitizer works as well in the real world as it does in laboratory testing (Johannes, 2013).

Alcohol-based hand sanitizer works against a variety of microorganisms but not spores. Some versions contain compounds such as glycerol to prevent drying of the skin (Boyce *et al*, 2002). Alcohol based hand sanitizers are proved to be the best for gastrointestinal and respiratory infections caused by viruses and gram negative bacteria (Boyce, 2002). The side effect of alcohol based hand sanitizer is its dryness of the skin. However it can be prevented by addition of humectants and skin conditioning agents (Mansour *et al*, 2013). Non-alcohol based hand sanitizer viz. benzalkonium chloride is known to have weak activity against gram negative bacteria as compared to alcohol and is prone to contamination by these bacteria (Armstrong, 1964). Alcohol has been used as an antiseptic at least as early as 1363 with evidence to support its use becoming available in the late 1800s (Stanton, 2001).

Several studies suggested that, sanitizers with at least 70% alcohol were suggested to kill 99.9% of the bacteria on hands (Rotter, 1999). However, the effectiveness of these sanitizers depends on the time of rubbing the sanitizer on hand. For instance, rubbing alcohol based sanitizers for 30 and 25 seconds was reported to kill 99.99% of bacteria on hand, respectively. It has been suggested that, the most important way to prevent the transmission of dangerous diseases is to frequently wash

hands with soap and water. If soap and water are not available it is recommended to use a hand sanitizer containing at least 60 percent alcohol to kill pathogenic bacteria on hand (Mercuy, 2009).

2.2 Microbes residing on the hands

Microbes residing on the hands are divided into resident and transient flora. Resident flora (e.g. *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus viridans*) colonizing deeper skin layers are more resistant to mechanical removal has lower pathogenic potential. Transient flora (e.g. *Staphylococcus aureus*, Gram negative bacilli, *Candida species*) colonizes the superficial skin layers for short periods, is usually acquired by contact with a patient or contaminated environment and these microorganisms are easily removed by mechanical means such as hand washing and are responsible for most health care-associated infections and the spread of antimicrobial resistance. The main pathogens of concern are Salmonella, *Staphlococcus aureus*, *Streptococi*, E.*coli*, and protozoa (Fazlar and Ekhtelat, 2012).

However, not everyone has frequent access to water and soap, which is why the more convenient hand sanitizers, which some companies claim to kill "99.9% of germs" (Purell, 2015) are increasing in popularity. Even the BCCDC recommends the use of hand sanitizers to supplement hand washing, which will increase its efficacy (BCCDC, 2014). The common opportunistic pathogens which cause hospital acquired infections are *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The most common sites through which these bacteria are transferred are the urinary tract, wounds, burns, blood, gastrointestinal tract etc. These bacteria in addition to the multidrug resistant bacteria are involved in nosocomial infections and the hands of health care workers are the primary mode of transmission of infection. Thus it is important for health-care workers to maintain proper hand hygiene to minimize the possible transfer of pathogenic agents (Iliyasu *et al*, 2016).

2.3 Natural herbs

Herbs are natural source used for treatment of various diseases. Also herbs are used for flavoring foods, gastronomic preparation, perfumery, cosmetics, beauty and body care. Many medicinal herbs are also food, oil and fiber plant (Peter, 2001). Herbs are rich in volatile oil which gives pleasant aroma. In addition, herbs may contain alkaloids and glycoside which have great pharmaceutical effect. Essential oils have been extensively investigated for their activity against a number of

storage fungi, plant and human pathogens, bacteria, insect, pests and other harmful microorganisms. Almost all essential oil of herbs and spices (individual or combination) are highly inhibitory to selected pathogenic and spoilage microorganisms (Kalemba and Kunicka, 2003). An essential oil is defined internationally as the product obtained by hydrodistillation, steam distillation or dry distillation or by a suitable mechanical process without heating (for Citrus fruits) of a plant or some parts of it (Rubiolo *et al*, 2010).

They are aromatic oily liquids, volatile, characterized by a strong odour, rarely coloured, and generally with a lower density than that of water. They can be synthesized by all plant organs (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and root) and therefore extracted from these parts, where they are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes (Burt, 2004, Bakkali, 2008). Essential oils only represent a small fraction of plant's composition; nevertheless they confer the characteristics by which aromatic plants are used in the food, cosmetic and pharmaceutical industries. The major constituents of essential oils are terpenes/ terpenoids and aromatic and aliphatic compounds, which are characterized as low-molecular-weight aroma chemicals (5–8) (Pourmortazavi and Hajimirsadeghi, 2007).

Generally, essential oils are comprised of two or three major components in relatively high concentrations (20–95%) and other components present in trace levels. Components with relatively high concentrations in essential oils are d-limonene (over 80%) in Citrus peel oils, carvacrol (30%) and thymol (27%) in *Origanum compactum* oil, α-/β-thujone (57%) and camphor (24%) in *Artemisia herba*-alba oil, 1,8-cineole (50%) in *Cinnamomum camphora* oil, α-phellandrene (36%) and limonene (31%) in *Anethum graveolens* leaf oil, carvone (58%) and d-limonene (37%) in Anethum graveolens seed oil, and menthol (59%) and menthone (19%) in Mentha piperita oil. Generally, these major components of essential oils determine their biological properties (Burt, 2004, Bakkali, 2008).

2.3.1 *Mentha spictata* (Spearmint) Leaf

The genus *Mentha*, one of the most important members of the Lamiaceae family, is represented by 19 species and 13 natural hybrids. *Mentha* species have been found to possess significant biological activities, including antimicrobial, anti-stomach, anti-vomitive, antiseptic, anti-infective, vermifuge, antitussive, digestive and diuretic. *Mentha spicata* (sperarmint) is the most common and popular mint which is widely grown in temperate areas of the world, particularly Africa, temperate Asia, and Europe, and is cultivated throughout all regions of the world. It is a perennial, rhizomatous and

glabrous herb that has a strong aromatic odor. It is used against cough and cold and as a diuretic and a spasmolytic (Karousou *et al*, 2007).

It has shown analgesic, antipyretic and anti-inflammatory effects as well. It is applied as breath freshener, antiseptic mouth rinse, and toothpaste. The fresh and dried iranian spearmint essential oils have been used as a flavoring agent in various food products, including cheese and doogh (Iranian yoghurt drink), chocolate, beverages, jellies, syrups, candies, ice creams, and chewing gum (Singh *et al*, 2015). Tongnuanchan and Benjakul reported that characterization of essential oils is commonly performed by GC-MS, through which small size of essential oil constituents (volatile compounds) can be abstracted according to their boiling points. Generally, the constituents in essential oils are hydrogenated and oxygenated terpenes (monoterpenes and sesquiterpenes), aromatic compounds like ketones, phenols, alcohols, methoxy derivative, coumarins and terpenoids (isoprenoids) (Tongnuanchan and Benjakul, 2014).

The essential oils extracted from *Mentha spicata*, worlwide distributed, were characterized by the chemotype couple carvone/limonene (15.3-78.76%)/ (5.3-22.31%) respectively (Hussain *et al*, 2010). 1,8-Cineole (6.84-17.0%) (Snoussi *et al*, 2015). menthone (18.6-21.9%), piperitenone oxide (24.0-79.2%), cis-carveol (21.3-24.3%), piperitone (22.17-28.16%), pulegone (26.71-72.1%), trans-β-caryophyllene (5.23-8.01%) and α-Humulene (0.1-29.9%) have also been reported as main components of *Mentha spicata* essential oils (Chauhan *et al*, 2011). Spearmint has long traditional medicinal use. It was taken as a tea to treat general di-gestive problems. Spearmint is widely used in commercially manufactured product, cooking and medicine for its aromatic and flavorsome qualities (Elumalai and Krishnappa, 2008).

2.3.2 *Citrus aurantifolia* Dulcis (Lime) leaf and fruit

The genus Citrus, belonging to the Rutaceae or Rue family, comprises of about 140 genera and 1,300 species. Citrus sinensis (Orange), Citrus paradise (Grapefruit), Citrus limon (Lemon), Citrus reticulate (tangerine), Citrus grandis (shaddock), Citrus aurantium (sour orange), Citrus medica (Citron), and *Citrus aurantifolia* (lime) are some important fruits of genus Citrus (Anwar *et al*.,2008). Until recently, health-promoting properties of citrus had always been associated with their content of Vitamin C. However, studies within the last decade have focused on identifying the bioactive compounds (Karousou *et al*, 2007). Some of the major classes of compounds in citrus include: flavonoids, limonoids, coumarins and phytosterols (Wang *et al*, 2007). Preliminary screening of *C. aurantifolia* fruit and other parts showed the presence of alkaloids, flavanoids, tannins, saponins,

steroids, cardiac glycosides, carbohydrates, phenols and reducing sugars (Wang *et al*, 2007, Dongmo *et al*, 2009).

Detailed analysis of C. *aurantifolia* using High-performance Lipid Chromatography (HPLC) and Gas chromatography Mass spectrometry have led to the identification of phytochemicals from the plant, which include: 2,4,6- trichloroanisole; 5-geranoxy-7-methoxy-coumarin; 6,7-dimethoxycoumarin; 8-geranoxypsoralen; 9,10-dimethyl-1,2- benxanthracene; a-bergamotene; a-phellandrene; a-pinene; aterpineol; b-bisabolene; b-caryophyllene; b-pinene, dlimonene; camphene; g-terpenene; p-cymene; apigenin; berfapten; bergamottin; ciral; citronellol; fenchol; germacrene B; imperatorin; isoimperatorin; isopimpinellin; isovitexin; kaempferol; kaempferol C. *aurantifoliattin*; limonene; nobiletin; o-cymene; oxypeucedanin hydrate; phellopterin; quercetin; rutin; sabinene; terpinolene (Khan and Abourashed, 2010, Loizzo *et al*, 2012).

Studies revealed that the extracts of root of *C. aurantifolia* have been found effective in inhibiting the growth of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Beta-haemolytic streptococci*, *Escherichia coli* and *Neisseria gonorrhoeae* (Aibinu *et al*, 2007). The fruit on the other hand was found to inhibit anaerobic facultative bacteria as well (Rahman *et al*, 2011). *C. aurantifolia* has been proved to have significant antimycobacterial activity especially against the isoniazid-resistant strain of Mycobacteria (Camacho-Corona *et al*, 2008).The antimycobacterial activity have been attributed to the presence of some phytochemicals in C. *aurantifolia* which include: 5, 8- dimethoxypsoralen, 5-geranyloxypsoralen, palmitic acid, linoleic acid, oleic acid, 4-hexan-3-one and citral (Nallely *et al*, 2012).

2.3.3 Aloe barbaddenis leaf

Aloe barbadensis is a permanent moist xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall. The innermost part of the leaf is a clear, soft, moist and slippery tissue that consists of large thin-walled parenchyma cells in which water is held in the form of a viscous mucilage (Newton, 2004). Therefore, the thick fleshy leaves of *aloe* plants contain not only cell wall carbohydrates such as cellulose and hemicellulose but also storage carbohydrates such as acetylated mannans (Tizard *et al*, 2004).

Aloe spp. has been used in traditional medicine, but it also exhibited various pharmacological properties according to the findings of modern pharmacological studies. Numerous in *vitro* and in *vivo* pharmacological studies as well as clinical trials have confirmed the traditional uses of *Aloe*

including wound healing and anti-ulcer activities. The study reports that *aloe barbaddenis* leaf have also indicated new properties such as anti-diabetic, hypoglycaemic, anti-cancer, antimicrobial, antiviral, antihyperlipidemic and anti-ulcer activities (Radha, and Laxmipriya, 2015).

GC-MS can provide meaningful information for components that are volatile, non-ionic, and thermally stable and have relatively low molecular weight. (Mansour *et al*, 2013) used micro propagated plant-lets of *Aloe barbaddenis* for evaluation of phytochemical components by GC-MS analysis. Several constituents from various phytochemical classes such as alkaloids, anthrones, chromones, flavonoids, glyocoproteins, naphthalenes and pyrones have been isolated from different *Aloe spp*. Aloin, aloesin, aloenin, aloeresin, aloe-emodin and chrysophanol are some examples of such bioactive compounds (Amoo *et al*, 2014). Although many biological investigations have been performed to reveal the active constituents of *Aloe spp*., it is still not yet completely clear which compounds are responsible for the various observed pharmacological properties. However, polysaccharide and glycoprotein (lectin) fractions of *Aloe spp*. have been reported to possess considerable bioactivity (Singab *et al*, 2015, Hamman, 2008).

Some important constituents such as Idazoxan, Dextroamphetamine,1- ethylpropylamine, Resepridone, P-methyl benzoic acid, 2-Thiophene carboxylic acid, Dimethyl 4-Chlorophenyl thiophosphate, Benzphetamine, Picloram and Jasmonoyl acid, Glyphosate, Mepyarmine, Physostigmine, Methylphosphonic acid, Selagiline, 2,4- Dicholorophenol was found (Lakhan *et al*, 2012). The antibacterial activity of emodin against *Escherichia coli* was proposed to be mediated through inhibition of solute transport in membranes. Many anthraquinones have shown antiviral and/or virucidal effects on enveloped viruses (Alves *et al*, 2004).

2.3.4 Ginger and its constituents

Ginger (*Zingiber officinale*), a member of the *Zingiberaceae* family, is a popular spice used globally especially in most of the Asian countries. Chemical analysis of ginger shows that it contains over 400 different compounds. The major constituents in ginger rhizomes are carbohydrates (50-70%), lipids (3-8%), terpenes, and phenolic compounds. Terpene components of ginger include zingiberene, β-bisabolene, α-farnesene, β-sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23-25%) and shogaol (18-25%) are found in higher quantity than others. Besides these, amino acids, raw fiber, ash, protein, phytosterols, vitamins (e.g., nicotinic acid and vitamin A), and minerals are also present (Demin and Yingying, 2010).

The aromatic constituents include zingiberene and bisabolene, while the pungent constituents are known as gingerols and shogaols. Other gingerol- or shogaol-related compounds (1-10%), which have been reported in ginger rhizome, include 6-paradol, 1-dehydrogingerdione, 6-gingerdione and 10-gingerdione, 4- gingerdiol, 6-gingerdiol, 8-gingerdiol, and 10-gingerdiol, and diarylheptanoids. The characteristic odor and flavor of ginger are due to a mixture of volatile oils like shogaols and gingerols (Ali *et al*, 2008). The chemical composition of essential oils of ginger has been identified and quantified by means of GC-MS or GC with flame ionization detector applications (Sultan *et al*., 2005; Singh *et al*., 2008).

According to Famurewa and Emuekele (2011), sliced oven drying at 50AB (SOD) of fresh ginger has the highest volatile oil, protein, calcium and magnesium (Famurewa *et al*., 2011). Gurdip Singh *et al*. (2008) extracted the essential oil of ginger (Zingiber officinale) by hydrodistillation method and subjected the oil to GC–MS analysis. Sultan *et al*. (2005) extracted the essential oil of Thai and Chinese ginger by means of steam hydrodistilation and carried out works concerning the composition of essential oils by GC with flame ionization detector (Sultan *et al*, 2005). Various analytical techniques employing chromatographic methods such as GLC and GC in conjunction with mass spectroscopy have been used to analyze pungent components in ginger by modifying the ginger extract and/or partially purified fractions to their trimethylsilyl (TMS) derivatives with a view to improve their volatility, stability and separation (Masada *et al*., 1973; Clark *et al*., 1977; Harvey, 1981; Masada *et al*., 1974).

More recently, He *et al*. (1998) described the analysis of ginger constituents by the combination of high pressure liquid chromatography with UV photodiode array detection and electrospray mass spectrometry (HPLC–UV–ESMS). Ginger (*Zingiber officinale*) is a medicinal plant that has been widely used all over the world since ancient times, for a wide array of unrelated ailments including arthritis, cramps, rheumatism, sprains, sore throats, muscular aches, pains, constipation, vomiting, hypertension, indigestion, dementia, fever and infectious diseases. Ginger has direct anti-microbial activity and thus can be used in the treatment of both bacterial and fungal infections. All Zingiberaceous plants have strong aromatic and medicinal properties and are characterized by their tuberous or non-tuberous rhizomes (Akintobi *et al*, 2013). Ginger is also used as a flavoring agent in foods and beverages and as a fragrance in soaps and cosmetics (Alam, 2013).

2.3.5 Chemical constituent of Ocimum cufudontii leaf

The aerial parts of *Ocimium* (*Labiatae* family) have been used in several traditional medicine systems to cure various diseases. *O. sanctum* is important constituent of many Ayurvedic cough syrups and expectorants. It helps to mobilize mucus in bronchitis, sore throat and asthma. In addition, antimicrobial, antioxidant, anticatarrhal, antispasmodic, anthelminthic, anti-inflammatory, immunomodulator, anti-stress, adaptogenic, cardioprotective, antiulcer, and anti-diabetic activities of extracts and chemical constituent of *O. sanctum* were reported. The advantage of this plant is that it is traditionally acceptable and considered as safe. However, *O. sanctum* shown to have antifertility and abortificient action in animal studies and it should be there for used cautiously in pregnant women (Andre *et al*, 2016).

Gas chromatography (GC)-mass spectrometry (MS) analysis of the essential oil revealed the presence of linalool (44.18%), 1,8-cineol (13.65%), eugenol (8.59%), methyl cinnamate (4.26%), iso caryophyllene (3.10%), and a-cubebene (4.97%) as the main components. Fresh leaves and stem of *Ocimum sanctum* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugeno. The essential oil showed potent anthelmintic activity in the caenorhabditis elegans model. The ethanolic extracts from the leaves showed better activity against the B-lactamase producing methicillin-resistant *staphylococcus aurens* strains. Alcoholic extract showed growth inhibition for vibrocholeral. Aqueousextract of the plant showed growth inhibition for *Klebsiella*, *Escherichia coli*, *proteus* and *staphylococcus aurens* (Uma and Devi, 2001).

It has significant ability to scavenge highly reactive free radicals. Antioxidant bioassay-directed extraction of the fresh leaves and stems of tulsi extract yielded: cirsilineol, cirsimaritin, isothymusin, isothymonin, apigenin, rosmarinic acid and appreciable quantities of eugenol. Eugenol is a major component of the volatile oil and other compounds also demonstrated good antioxidant activity (Kalpesh Bhatt, 2012). The essential oils of *O. tenuiflorum* have been reported to possess methyl eugenol (Joshi, 2013a), β-caryophyllene (Kothari *et al*, 2005), (E)-caryophyllene, eugenol and, β-elemene (Awasthi & Dixit, 2007), methyl chavicol, and linalool , β-bisabolene, 1,8-cineole and methyl chavicol (Kicel, Kurowska, & Kalemba, 2005), and isocaryophyllene (Gbolade & Lockwood, 2008); eugenol, β-caryophyllene and caryophyllene oxide,eugenol, β-elemene and β-caryophyllene, methyl chavicol, camphor and β-caryophyllene (Khan *et al*., 2010).

3. Material and method

3.1 Chemicals and reagents used in this study

Nutrient Agar (For bacterial cultivation) was purchased.

Glycerine. It serves as an excellent emollient, moisturizer and lubricant to prevent skin dryness in personal care products.

Deionized water: Used as a solvent.

Menta spictata (Spearmint) essential oil: Used as a source of essential oils for flavoring, Perfumes and has been used as a valuable source of the effective antioxidant.

Alcohol: It is used as active antimicrobial ingredient in appropriate concentration and as solvent.

Aloa Leaf: A natural emollient and skin conditioner.

Citrus aurantifolia Dulcis (Lime) leaf oil: Function in hand sanitizer as fragrances and/or skin conditioning agents and disinfectant or act as an antioxidant and environmentally kindly cleaning agent and sequestering agents.

Other group of ingredients: It includes perfumes, vitamins etc.

3.2 Instruments and apparatus

Equipment's used for study were pH meter, analytical balance, Brookfield Viscometer, equipment for Distillation, alcohol meter ,rotary evaporator and heating mantle.

3.3 Collections of Plant

Mentha spictata and *Ociumium Cufodontii* were collected from Asela, Oromiya region, 75km from Adama. *Ginger* was purchased, *Aloe barbbaddenis* leaf and *Citrus aurantifolia Dulcis* (Lime) leaf were collected from Adama city. The plant materials were identified by Melaku Wondafrash Department of biology,Addis Abeba University,Ethiopia.

3.4 Extraction

3.4.1 Extraction of spearmint oil from *Mentha spictata* (spearmint) leaf

Distillation of spearmint oil was carried out using the method reported in litrature (Harborne, 1984). Freshly grounded leaf (145g) sample was weighed in 500ml rounded bottom capacity flask. Then it was filled with distilled water and fitted to Clevenger receiver and condenser. It was heated at 80 ºC using heat mantle for 2hrs till the volume of oil at the top of the receiver became constant. Oil was then collected and stored in desirable container till used. The extraction process was done repeatedly to obtain excess volume of oil.

3.4.2 Extraction of lime essential oil from *Citrus aurantifolia dulcis* fruit and leaf

Citrus fruit (96.6 g) was grounded and placed into a 500mL round bottom flask containing 250mL of distilled water. Then, the flask was connected to a Clevenger set up. Distillation was carried out for 2hrs at 80^{o}c. The essential oils evaporated with steam during the distillation process, and was separated from the condensates and collected in the distillate receiver. After collection it was placed in a desired place (Kawamoto *et al*, 2013). The extraction process was done repeatedly to obtain excess volume of oil.

3.4.3 Water and ethanol extraction of Ginger, *Aloe* leaf and *Ocimum cufudontii*

Water and ethanol extract of *ginger*, *Aloe and Ocimum* leaf were extracted by Maceration process (Fatima *et al*, 2015). Leaves of the plants were washed, dried, grounded, measured and soaked in ethanol 1.5L for 72hr with 24hr gap shaking for each plant materials. The extract was filtered by What mann no.1 filter paper. The ethanol in the filtrate was evaporated by rotary evaporator at 40^{o}c at 90rpm. The residue was again soaked by 1.5L of distilled water for 72hrs.The amount of ethanol content within the extracts was measured by alcohol meter Gay Lussac at 15^{o}c. Then after it was stored in preferred place till used for further experiments.

3.2 Selection of test organism

Pathogens used for evaluation of anti-microbial activities of extract and formulated hand sanitizer were: *Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC-22953), Entrococcus feasilis (*ATCC 229212*), P. aeroginosa (*ATCC 27853*), Klebsiella pneumonia (*ATCC 700603) and

Shigella sonnie (ATCC 2593).The strains were obtained from Oromia public health research capacity building and quality assurance laboratory.

3.6 Determination of the antimicrobial activities of the plant extracts

During the process of antimicrobial testing, each plant extract were subjected to antimicrobial screening by using disc diffusion method against the selected bacterial strains. The nutrient agar were prepared for each test organism following standard procedure reported in literature (Clutterbuck *et al.*, 2007). 20ml of sterile MHA (maintained at 45-50^{o}C in a molten state), poured into sterilized Petri dishes. After solidifying, standardized inoculum, well-isolated colonies of the same morphological type of bacteria were selected and spread on MHA plate. Then previously prepared four filter paper discs were placed onto the surface of the agar plate at equal distance from each other and 15 mm from edge of plate. Each disc were pressed down to ensure complete contact with the agar surface and the discs were placed with a dispensing apparatus, they distributed evenly. Ordinarily, 4 discs were placed on each plate. Each plate were inverted and placed in an incubator set at 37^{0}C for 24 hrs after the discs are applied for bacteria. The antibacterial susceptibility was evaluated by measuring zone of inhibition using plastic ruler in mm. The inhibition zones produced by the formulated polyherbal hand sanitizer plant extracts were compared with the inhibition zones produced by commercial hand sanitizer. Ceftriaxone drug 5 µg/disc was used as positive control.

3.7 Formulation of polyherbal hand sanitizer

3.7.1. Formulation of alcoholic based polyherbal hand sanitizer

Procedure

Isopropanol alcohol (5%) was added to deionized water with slow stirring. Citric acid (0.5%), sodium lauryl sulphite (1%), Glycerin (30%) were added respectively, with continuous stirring with glass rod stirrer. 10drops of perfume and 1% of vitamin E were added and mixed with slow stirring. The solution was filled to mark of 1000mL Beaker by distilled water and homogenized by stirring to obtain uniform product (Table1).

Table 1. Formulation of hand sanitizer without plant extract

Sample code	Ingredient	Quantity taken
HS1	Isopropanol alcohol	5%
	sodium lauryl sulphite(SLS)	1%
	Glycerin	30%
	Citric acid	0.5%
	Distilled water	63.45%
	Vitamin E	1%
	Perfume	10 drop

3.7.1.1 Formulation of polyherbal hand sanitizer with different composition of water and ethanol plant extract.

Procedure

2%, 5%, 10% and 20% of water and 2%, 5% and 10% of ethanol extract were prepared in 100mL different beakers. The ingredient and quantity prepared in the formulation of table 1 was used for each formulation. The solution were mixed by stirring with glass rod stirrer. Spearmint essential oil and Lime essential oil were added to each preparation for flavoring (Table 2). The mixture were mixed in order to make uniform product. Sample without plant extracts was also prepared in another 100mL beaker.

Table 2. Formulation of polyherbal hand sanitizer with different composition of water and ethanol plant extract.

Ingredients	**Sample quantity**						
	Water extract				**Ethanol extract**		
	HS2	HS3	HS4	HS5	HS6	HS7	HS8
Ginger, *Aloe*, *Osmium* water extract and Spearmint and Lime essential oils	2% each	5% each	10% each	20% each	2% each	5% each	10% each

3.7.1.1.1 Optimization for formulation of alcohol based polyherbal hand sanitizer

Formulation with glycerin as major composition within 5% plant extracts was carried out to improve the viscosity of the formulated hand sanitizers (Table 3).The formulation with 60%isopropanol alcohol was carried out to evaluate the anti-bacterial activities of alcohol when

compared with plants antibacterial activities. The formulation also done with 1% SLS and without SLS to reduce the foam producing ability of the formulated sanitizers (Table 4). This is because of the hand sanitizer is targeted for only anti-bacterial activities and conditioning the hand.

Table 3. Optimization carried out with glycerin

Sample code	Ingredient	Quantity taken
HS9	Isopropanol alcohol	5%
	sodium lauryl sulphite(SLS)	1%
	Glycerin	50%
	Citric acid	5%
	Lime essential oil.	Two drop
	Spearmint essential oil	Two drop
	Perfume	Two drop
	Vitamin E	1%
	Distilled water	20%

Table 4. Formulation carried out by isopropanol alcohol as major component without plant extract and SLS

Sample code	**Ingredients**	**Quantity taken**
HS10	Isopropanol alcohol	60%
	sodium lauryl sulphite(SLS)	1%
	Glycerin	30%
	Citric acid	2%
	Lime essential oil.	Two drop
	Spearmint essential oil	Two drop
	Perfume	0.05%
	Vitamin E	1%
HS11	All HS10 Without SLS	Without 1%SLS

3.7.2 Formulation of alcohol-free polyherbal hand sanitizer

Procedure

1% of sodium lauryl sulphite was added to deionized water with slow stirring to avoid formation of possible air bubbles. Citric acid (0.5%), Glycerin (30%) were added respectively with continuous stirring with glass rod stirrer .The solution was filled to mark by distilled water and homogenized by stirring. 0.5% of perfume was added and mixed with slow stirring to obtain uniform product (Table 5).

Table 5. Formulation of alcohol-free hand sanitizer without plant extract

Sample code	Ingredient used	Quantity taken
HS12	Sodium lauryl sulphite	1%
	Glycerin	30%
	Citric acid	0.5%
	Perfume	0.5%
	Vitamin E	1%
	Distilled water	67%

3.7.2.1 Formulation of alcohol-free polyherbal hand sanitizer at different composition of plant extracts.

Procedure

In this formulation the prepared ingredient and quantity in Table 5 was used. The formulation was carried out in 100mL different beakers (Table 6). 2%, 5%, 10% and 20% of each plant extract were prepared in different beakers. Then the formulation were filled up by preparation in table 5.

Table 6. Formulation of alcohol-free polyherbal hand sanitizer at different composition of plant extracts.

Ingredients	Sample cod and quantity added			
	HS13	HS14	HS15	HS16
Ginger, *Aloe*, *Osmium* water extract and Spearmint and Lime essential oils	2% Each	5% each	10%each	20%each

3.8 Evaluation of formulated polyherbal hand sanitizer

3.8.1 physico-chemical evaluation

3.8.1.1 Physical appearance/visual inspection

The formulation prepared was evaluated for the clarity, color, odor, foam producing ability, Homogeneity and Appearance (Shaloo *et al*, 2017). Clarity, homogeneity and color was checked by necked eyes against white background and the odor was smelled.

3.8.1.2 Determination of pH

The pH values of all prepared formulations were determined by using digital pH meter. The measurement was done in previously calibrated pH meter at room temperature (25°C).

3.8.1.3 Determination of % of solid contents

Good hand sanitizer usually has an appropriate amount of solid content as it is easy to be applied and rinse out from hand. 4 grams of hand sanitizer was placed in a previously clean, dry and weighed evaporating dish. The dish and sanitizer were weighed again to confirm the exact weight of the sanitizer. The liquid portion of the sanitizer was evaporated by placing the evaporating dish on hot plate. The weight and thus % of the solid contents of sanitizer left after complete drying was calculated.

3.8.1.4 Dirt dispersion test

Two drops of the prepared sanitizer were added to 10 mL of distilled water taken in a large test tube. To this solution, one drop of India ink was added and the test tube was stoppered and shaken ten times. The amount of ink in the foam indicated by the rubric such as None, Light, Moderate or Heavy (Ali and Kadhim, 2011).

3.8.1.5 Viscosity evaluations

The viscosity of Poly herbal hand sanitizer was determined using digital Brookfield viscometer at 100 rpm (Padasalgi *et al*, 2008).

3.9 Antibacterial testing for formulated hand sanitizer

Antimicrobial testing of the prepared formulation of each polyherbal hand sanitizer were subjected to antimicrobial screening using disc diffusion method against the selected organisms. 5µl of each formulated sanitizer were used for evaluating the antimicrobial activities. The plates were incubated at 37^0C for 24 hours and the zones of inhibition were recorded .All the tests were repeated 3 times for precise results.

3.10 Data analysis

All tests was performed in triplicate and data were expressed as Mean ± standard deviation.

4. Result and discussion

4.1 Physical evaluation of formulated hand sanitizer

Physicochemical parameters of prepared hand sanitizers were determined. Parameters such as color, odor, appearance, viscosity, pH, percentage of solid content, dirt dispersion ability and stability were tested. The formulation exhibit good appearance characteristic as well as the general characterization of the sanitizer proved that the formulations do not show any unpleasant color and odor (Fig 1below)

Figure 1. Formulated polyherbal hand sanitizer from plant

The pH was found in the range of 6.8-7.99 which is in mild acidic and neutral range. The pH of Hand sanitizer has been shown to be important for improving and enhancing the qualities of hand, minimizing irritation to the hands and stabilizing the ecological balance of the skin. The current trend to promote hand sanitizer of mild pH is one of the ways to minimize damage to the skin. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine (Nandkishor, 2013).

The percentage of solid content of formulated sanitizer is between 0.373-3.29% which is very low and can be simply applied to hand and rinsed away during hand washing. The formulated sanitizer also tested for the stability of foam that shows small height of foam (1cm for formulation with 1% of SLS and 0.5cm for formulation without SLS) and the stability of foam lost after 1min. This shows that the formulated hand sanitizer has small foam producing ability and only used for antibacterial activity. The viscosity measured was in the range of 27-105cps with optimization shows 105cps viscosity. This shows that the formulated polyherbal hand sanitizer exists in liquid form when compared to the reference hand sanitizer from market which is in gel form and have 364cps viscosity (Table 7 and 8).

Table 7. Physico-chemical characteristics of alcohol based hand sanitizers

Parameter	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8
Odor	Perfumed	Aromatic	Aromatic	Aromatic	Aromatic	aromatic	aromatic	aromatic
pH	6.81	7	7.32	7.4	7.89	6.85	7.06	7.45
Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	uniform
Appearance	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Viscosity(cps)	46	33.33	30.33	28.83	27.32	44.33	36	32
Color	Colorless	light red	light red	light red	light red	Red	brownish	brown

Table 8. Parameter evaluation for sample optimized and alcohol free poly herbal hand sanitizer

Parameter	HS9	HS10	HS11	HS12	HS13	HS14	HS15	HS16
Odor	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant	Fragrant
pH	7.99	7.79	6.80	6.76	7.06	7.6	7.75	7.99
Homogeneity	Uniform	Uniform	Uniform	Uniform	Uniform	Uniform	uniform	uniform
Appearance	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid	Liquid
Viscosity	105	41	36	44.33	27	43	54.33	72.67
Colour	White	Light red	Light red	Colorless	Light red	Light brown	Light brown	brown

4.2 Result of Antibacterial activities of plant extracts and formulated polyherbal hand sanitizers

4.2.1 Antibacterial activity of plant extracts

The antibacterial activity of each plant extract were determined by disc diffusion method against six selected strains of bacteria, the result of each measured zone of inhibition in mm was recorded in Table 9 below. The result shows that the ethanol extract of *aloe* inhibits the growth of two bacterial species *Shigella sonnie* (ATCC 25931) and *Enterococcus feasilis*, ATCC 229212*), O.cufudontii* against four bacterial species *Escherichia coli(*ATCC 25922), *Staphylococcus aureus (*ATCC 25923*), P.aeuroginosa (*ATCC 27853*) and K. pneumonia(*ATCC 700603*),*Ginger against one

bacterial species *Entrococcus feasilis (*ATCC 229212*),*lime essential oil against four bacterial strains *Shigella sonnie (*ATCC 25931*), Escherichia coli (*ATCC 25922*),Entrococcus feasilis (*ATCC 229212*) and Staphylococcus aureus (*ATCC 25923*) and* spearmint essential oil shows antibacterial activities against *Shigella sonnie* (ATCC 25931).This indicates that all the plant extract can inhibited the growth of one or more of the selected pathogenic bacterial species which confirms the literature for this study. The growth of bacterial organism on some crude extracts can be due to natural resistance of the organism or lack of antimicrobial principles. Thus plants are considered as the greatest source to obtain new antimicrobials.

Table 9. Antibacterial activity of plant extracts**.** (Mean ± SD) (n= 3).

Organism	**Zone of inhibition of plants extract (mm)**				
	Aloe	*Ocimum*	*Ginger*	Lime oil	Spearmint oil
Shigella sonnie (ATCC 25931)	8±1	-	-	9±1	7±1
*Escherichia coli (*ATCC 25922)	-	7.67±1.97	-	8±2	-
*Entrococcus feasilis (*ATCC 229212*)*	9±1.87	-	9±1	8.67±0.34	-
*Staphylococcus aureus (*ATCC 25923*)*	-	18±1	-	11.67±2.3	-
*Pneudomonas(*ATCC 27853*)*	-	8±1	-	-	-
*Klebsiella pneumonia(*ATCC 700603*)*	-	9±2	-	-	-

4.2.2 Antibacterial activity of formulated polyherbal hand sanitizer

In this case of study alcohol-free and low percent (5% isopropanol) alcohol-based polyherbal hand sanitizer were formulated. The optimization was also carried out to 60% isopropanol alcohol with 5% plant extract as major components. This is prepared to determine the anti-microbial effectivity of plant extracts in the formulation when composition of alcohol is changed. This helps to compare the effectivity of plant with isopropanol alcohol which have antimicrobial properties against microbes. The antibacterial activity of each formulation were determined using disc diffusion method against six selected strains of bacteria, the result of each measured zone of inhibition in mm was recorded in table forms.

4.2.2.1 Result of antibacterial activity of alcohol-based polyherbal hand sanitizers

The result shows that, the antibacterial activity of alcohol based hand Sanitizer without plant (HS1) showed inhibition of bacterial growth against three bacterial species (*E. coli*, *S. aureus* and

enterococcus feasils) whereas hand sanitizer within 2% of water plant extract (HS2) against four bacterial species (*Shigella sonnie, E. coli, S. aureus* and *enterococcus feasils).* Hand sanitizer within water plant extract of 10% (HS4), 20 %(HS5) and hand sanitizer within 2% (HS6) ethanol extract showed antibacterial activity against three bacterial species (*E. coli, S. aureus* and *enterococcus feasils)* with zone of inhibition observed in Fig 2 below.

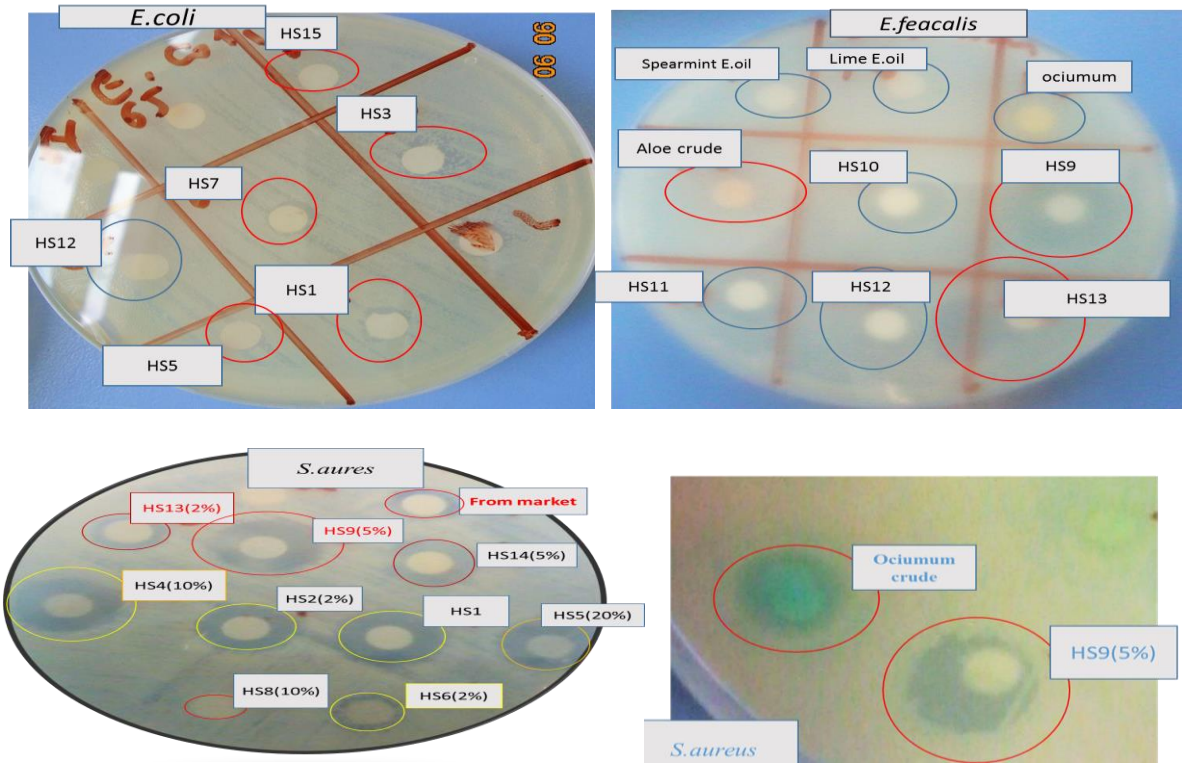

Figure 2. Result of observed zone of inhibition for formulated polyherbal Hand-sanitizer and plant extract against selected pathogens

Hand sanitizer with 5% of ethanol plant extract (HS7) formulation showed antibacterial activity against five species (*Shigella sonnie*, *E. coli*, *S. aureus*, *Enterococcus feasils*, *Pneudomonas*). The hand sanitizer with 10% ethanol extract (HS8) showed antibacterial activity against three bacterial species (*Shigella sonnie, S. aureus* and *enterococcus feciialis).* Hand sanitizer with 5% of water plant extract (HS9, HS10) showed promising antibacterial activity against the selected bacterial species (*Shigella sonnie, E. coli, S. aureus, Enterococcus feasils, pneudomonas and K.pneumonia*) compared to the reference hand sanitizer from market. As observed from the Fig 2 above the highest activity was observed for HS9 against *Staphylococcus aureus* with zone of inhibition 23±2.08mm shown in Table 10 below. This results shows that the activity of the polyherbal hand sanitizer formulated with 5%isopropanol (HS9) is effective than the formulation optimized to 60%

isopropanol alcohol (HS10) in which its zone of inhibition shows 14±1mm with major component of 5% plant extracts.

Table 10. Antibacterial activities of alcohol based polyherbal hand sanitizers.

Organism	**Zone of inhibition and sample code**					
	HS1	HS2	HS3	HS4	HS5	HS6
Shigella sonnie (ATCC 25931)	-	8.33±0.57	9±1	-	-	-
*Escherichia coli (*ATCC 25922)	9±2.12	6.67±0.58	7±1	6.67±0.17	7.33±0.67	7
*Entrococcus feasilis (*ATCC 229212*)*	11.33±1.15	7.33±1.53	10.33±0.18	9.33±1.17	10.33±1.17	13±1
Staphylococcus aureus (ATCC 25923)	13±2.65	11.67±2.3	12±1	9.67±0.67	9±1	11±1
*Pneudomonas(*ATCC 27853*)*	-	-	-	-	-	-
*Klebsiella pneumonia(*ATCC 700603*)*	-	-	-	-	-	-

Organism	**Zone of inhibition in mm and sample code**					
	HS7	HS8	HS9	HS10	HS11	**Market hand sanitizer**
Shigella sonnie (ATCC 25931)	11.67±1.17	12.33±2.17	13±1	10.67±0.17	-	-
*Escherichia coli(*ATCC 25922)	7±1	-	7±1	10	8	-
*Entrococcus feasilis (*ATCC 229212*)*	9	10±1	16±2.65	14.33±1.17	12.67±0.67	-
Staphylococcus aureus (ATCC 25923)	12±1	7±1	23.33±2.08	14±1	-	7±1
*Pneudomonas(*ATCC 27853*)*	-	-	15±1	15±0.17	-	-
*Klebsiella pneumonia(*ATCC 700603*)*	-	-	-	12.34±0.18	8±1	-

4.2.2.2 Antibacterial activity of alcohol-free hand sanitizer

Alcohol-based antiseptic formulation preparations may are flammable and do not demonstrate persistent antimicrobial activity. Also, repeated use often can cause drying and irritation of the skin. Alcohol strips the skin of essential oils and sebum, which act as a natural protective barrier against bacterial infection and precipitate protein. Together, these and other adverse properties greatly limit

the alcohol-based antimicrobial product's immediate effectiveness and increase the chances for the spread of infection (Paulson, 1994). Thus the alcohol-free hand sanitizer in this study was needed to be formulated to observe the contribution of antibacterial activities of plant extracts without alcohol in formulated hand sanitizers.

The result indicates that alcohol-free hand sanitizer with 2% plant extracts (HS13) showed antibacterial activity against two bacterial species *Escherichia coli (*ATCC 25922) and *Staphylococcus aureus* (ATCC 25923)). While Hand sanitizer with 5 %(HS14), 10 %(HS15) and 20 %(HS16) plant extracts shows antibacterial activity against two bacterial species (*S. aureus*, *Enterococcus feasils*). The highest activity was observed for HS13 against *Staphylococcus aureus* with its zone of inhibition shown in Table 11 below. The formulation were preferable and effective when compared to reference hand sanitizer taken from market. But the formulated alcohol-free hand sanitizer fails to inhibit the growth of some of the selected bacterial strains (*Klebsiella pneumonia (*ATCC 700603) and *Pneudomonas auregeuosa (*ATCC 27853*)* except for the formulation without plant extract inhibit the growth of *Klebsiella pneumonia* regarding to alcohol-based hand sanitizers formulated.

Table 11. Antimicrobial activities of alcohol-free polyherbal hand sanitizer

Organism	**zone of inhibition in mm and sample code**				
	HS12	HS13	HS14	HS15	HS16
Shigella sonnie (ATCC 25931)	-	-	-	-	-
*Escherichia coli(*ATCC 25922)	-	7±1	-	-	-
*Entrococcus feasilis (*ATCC 229212*)*	10±1	-	8.33±0.67	7	7
Staphylococcus aureus (ATCC 25923)	7±1	10±1	8	9.67±0.3	7±1
*Pneudomonas auregeuosa(*ATCC 27853*)*	12.67±0.67	-	-	-	-
*Klebsiella pneumonia(*ATCC 700603)	-	-	-	-	-

5. Conclusion and Recommendation

This study generally conclude that, the possible finding was carried out to formulate poly herbal hand sanitizer from plant extracts which found to have antibacterial activities and effective when compared to that of reference hand sanitizer obtained from market.

From the result, the physicochemical evaluation indicates that, the formulation exhibit good appearance characteristic as well as the general characterization of the sanitizer proved that the formulations do not show any unpleasant color and odor. It was also inferred from the results that the formulations which was prepared by 50%glycerin in co-operation were found to be good in viscosity.

The observed anti-bacterial activity results demonstrate that all formulated poly herbal hand sanitizer and plant extract can inhibited the growth of one or more of the selected pathogenic bacterial species which confirms the literature of this study. The growth of bacterial organism on some crude extracts and formulated sanitizers can be due to natural resistance of the organism or lack of antimicrobial principles. Thus plants are considered as the greatest source to obtain new antimicrobials. The easy accessibility of plants and their effectiveness helps to manufacture with cost effective benefits.

The reference hand sanitizer taken from market which includes ingredients such as carbomer, aqua deem, ethanol (60%) and fragrance showed antibacterial activity against *Staphylococcus aureus* only with zone of inhibition shows 7±1mm (as shown Fig 2 of this paper) and fails to inhibit bacterial growth for most strains of microorganisms when compared to that for the formulated hand sanitizer in this project. This proves that the formulated alcohol and alcohol-free polyherbal hand sanitizer is preferable and effective. Hand sanitizer having 5% plant extracts and 5% isopropanol alcohol was found to be more effective with its zone of inhibition shows 23.33±2.08mm, when compared to 60%isopropanol alcohol which has 14±1mm zone of inhibition. This shows that polyherbal extract is more effective than isopropanol which has antimicrobial properties.

This study recommends that the formulated sanitizer is in liquid form which has moderate viscosity compared to commercial hand sanitizer and viscosity can be adjusted by carbomer to form viscous hand sanitizer. Further work is also recommended to conduct the toxicity analysis of formulated hand sanitizers before applied to hands. The easy accessibility of plants and their effectiveness also helps with cost effective benefits, harmless thus further study should also carried out against green medicinal plants to use traditional plant for different purposes.

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