



Formulation and Evaluation of Herbal Sanitizer by using Natural Herbs

Sakshi Rastogi¹, Darshika Sharma², Sakshi Gupta³

¹S.D College of Engineering and Technology, Muzaffarnagar

²Assistant Professor, Shri Ram College, Muzaffarnagar

³Business Development Executive, Mellalta meets LLP, Delhi

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Abstract

The main aim for the preparation of herbal hand sanitizer is to maintain the good hygiene and also promotes traditional use of herbs as an alternative to chemicals which are used in the formulation of sanitizers. The antimicrobial test was carried out using dip-well method in which each plant extract i.e. *Azadirachta indica*, *Ocimum sanctum*, *Allium sativum*, *Cymbopogo*, *Cinnamomum camphora*, *Terminalia arjunaw* was individually tested against *Shewanella putrefaciens*, *Bacillus cereus*, *Salmonella spp.* The one which forms the largest zone of inhibition was taken for the formulation of herbal sanitizer. The efficacy of the prepared herbal hand sanitizer was checked/ evaluated by finger impression method and the results were compared with free hand, ordinary soap, formulated herbal hand sanitizer, alcohol formulated herbal hand sanitizer impressions on nutrient agar media plate.

The results showed that alcohol based formulated herbal sanitizer had lot of reduction in number of colonies whereas formulated herbal sanitizer has also reduced significant number of colonies. Thus, owing to higher antimicrobial activity and efficacy these herbal extracts can be used in the preparation of herbal hand sanitizers on commercial scale, which will not cause any directly or indirectly ill effects on humans as chemical based sanitizer does.

Keywords: Herbal Sanitizer, Antibacterial, Antimicrobial

Introduction

Everybody needs to maintain the hygienic conditions around them. But sometimes it can't be possible because in the environment we are living is exposed of microbes and many of them are pathogenic. Skin is the first most exposed part of the body which needs protection^[1]. Hands perform the majority of functions of the human's body and are exposed to a variety of substances which include soil during farming, food during cooking, touching raw and contaminated food material, during personal hygiene^[2]. Right hand washing and drying is the key method to stop the transmission of pathogens from hand to another

part of the body. Hand Sanitizer is a supplement or alternative to hand washing with soap and water. Many preparations are available, including gel, foam, and liquid solutions. The active ingredient in hand sanitizers may be isopropyl alcohol (isopropanol), ethanol, n-propanol, or povidone-iodine. Inactive ingredients in alcohol rubs typically include a thickening agent such as polyacrylic acid for alcohol gel, humectants such as glycerine for liquid rubs, propylene glycol.

*Corresponding Author: Darshika Sharma

When hands are not visibly dirty, the United States centres for disease control and prevention and many other world public authorities recommend hand sanitizers as an acceptable alternative to soap and water for health hygiene. There are many sanitizers are available in the market and they contain many harmful chemicals which may cause skin irritation and also resistant among pathogens. The Hands of health care workers are the primary mode of transmission of these multi drugs resistant pathogens and infections to patients through proper hand hygiene is the single most important simplest and least expensive means of preventing health care associated infections and the spread of antimicrobial resistance but unfortunately poor hand hygiene practices are observed due to lack of scientific knowledge, unawareness of risks, misconceptions(e.g. Glove used obviates the need for hand hygiene), unavailability of hand hygiene facilities, under staffing and patient overcrowding [3].

Organism such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Bacillus subtilis*, *Candida albicans*, *Salmonella* species cause wide variety of skin infection. Historically, plant provides a good source of ant infective agent. Plant based antimicrobial represent a vast untapped source from medicine. They are effective in the treatment of infectious disease. Hand washing removes visible dirt from hands and reduce the number of harmful microorganisms. Harmful bacteria and viruses such as *E. coli* and *Salmonella* can be carried by people, animals or equipment and transmitted food. One study showed that proper hand washing and other simple procedures can decrease the rate of catheter related blood stream infections by 66% [4]. Plant contains flavonoids and polypeptides used in the traditional medicine have been found to be effective against wide variety of infections. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids which have been found in vitro to have antimicrobial property [5]. So, there are prerequisite to develop herbal based sanitizer which is useful against pathogens and doesn't create any skin hazard.

There are many sanitizers are available in the market and they contain many harmful chemicals

which may cause skin irritation and also resistant among pathogens^[6] There are still some microbial species like *Bacillus* species, *Shewanella* species which causes skin diseases and infection and required attention.

Selection of natural material/ plant species Following plants are selected as for the formulation of herbal sanitizer.

- 1- Neem leaves (*Azadirachta indica*)
- 2- Garlic (*Allium sativum*)
- 3- Lemon grass (*Cymbopogon*)
- 4- Basil leaves (*Ocimum sanctum*)
- 5- Camphor (*Cinnamomum camphora*)
- 6- Arjuna (*Terminalia arjuna*)

Why it has been chosen:

Neem (*Azadirachta indica*) Products made from neem trees have been used in India for over 2 millennia for their medicinal properties because of the anthelmintic, antifungal, antibacterial, antiviral constituents of the neem tree^[7-9].

The process of extracting neem oil involves extracting the water insoluble components with ether, petrol, ethyl acetate, dilute alcohol, methanol. Nimbidin is the main antibacterial ingredient^[10].

Garlic (*Allium sativum*)

As powerful antibiotics lose their punch against "superbugs" such as methicillin-resistant *Staphylococcus aureus* (MRSA), scientists are searching for new antimicrobial agents from natural sources. Allicin, the major component of garlic, is one such agent, and it was recently shown to be potent against MRSA. Allicin, one of the active principles of freshly crushed garlic homogenates, has a variety of antimicrobial activities. Allicin in its pure form was found to exhibit

- 1- antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria,
- 2- antifungal activity, particularly against *Candida albicans*;
- 3- ant parasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and
- 4- Antiviral activity.

The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol dehydrogenase, thioredoxin

reductase, and RNA polymerase, which can affect essential metabolism of cysteine proteinase activity involved in the virulence of *E. histolytica*.

Lemon grass (*Cymbopogon*) Research shows that lemongrass oil has antifungal properties. The defensive antioxidant activity of the lemongrass herb protects against antibiotic-kaempferol, elimicin, catecol, chlorogenic acid, and caffeic acid, all of which help in providing an impressive range of medicinal aids. The main component of lemongrass is lemonal or citral, which has anti-fungal and antimicrobial qualities, while also providing a distinct lemony smell.

Basil leaves (*Ocimum sanctum*): Even going close to a Tulsi plant alone can protect you from many infections. Tulsi's unique combination of antibacterial, antioxidant and anti-inflammatory and analgesic activities also makes it useful in wound healing^[11]. Keeping in view the ultra-disinfectant and germicidal properties of this legendary herb, wise people then devised these customs to bring people into contact with this plant every day, so that they may keep safe from day-to-day infections. Fever is mainly caused due to infections from protozoa (malaria), bacteria (typhoid), viruses (flu) and even allergic substances and fungus. It's mostly used due to the presence of components like Camphene, Eugenol and Cineole in its essential oils.

Camphor (*Cinnamomum camphora*): Camphor oil (*Cinnamomum camphora*) is a potent essential oil that brings a number of health benefits. Camphor is readily absorbed through the skin, producing either a coolness or warmth sensation^[12]. Ironically, camphor oil is now widely recognized in the aromatherapy field even though it's a common ingredient in mentholated products or ointments to help ease skin diseases and fungal infections. The major components of camphor oil are alcohol, borneol, pinene, camphene, camphor, terpene, and safrole. Camphor is also used topically as an eardrop, and for treating minor burns. Camphor seems to stimulate nerve endings that relieve symptoms such as pain and itching when applied to the skin. Camphor is also active against fungi that cause infections in the toenails

Arjuna (*Terminalia arjuna*): It is a large sized 20-25 m deciduous tree having buttressed trunk having lot of medicinal properties. The bark of *Terminalia arjuna* is soft and thick with grey

colour on the outer surface and tinge inside. It has many medical properties such as in maintaining the cholesterol level at the normal rate. Many useful phytoconstituents have been isolated from *T. arjuna* which included triterpenoids for cardiovascular properties, tannins and flavonoids for its anticancer, antimicrobial properties and so on^[13]. Strengthen the heart muscle so maintain the good heart functionality in the body. It also has antimicrobial property. The bark of the *Terminalia arjuna* constitutes an important crude drug, which contains tannins, triterpenoids saponins, flavonoids, sterols, calcium salts, alkaloidal and glycosidal substances, arjunine and arjunglycoside etc. Arjunolic Acid 0.5%, Tannin 25%.

Plant Material Used

Plants name	Part of the plant taken
<i>Azadirachta indica</i>	Leaves
<i>Ocimum sanctum</i>	Leaves
<i>Cymbopogon</i>	Leaves
<i>Allium sativum</i>	Seeds
<i>Cinnamomum camphora</i>	Leaves
<i>Terminalia arjuna</i>	Bark

Table no. 1 showing plant material taken for the study of antimicrobial activity

Plants were collected from the sheetal nursery located on the bhopa road, Muzaffarnagar, Uttar Pradesh. The microorganisms used in this study were *Shewanella putrefaciens*, *Bacillus cereus*, *Salmonella spp.* Out of which two bacterial species ie *Shewanella putrefaciens* and *Bacillus cereus* were collected from the MTCC (The Microbial Type Culture Collection, Chandigarh) and *salmonella spp.* from the Lal Pathology, Muzaffarnagar.

Shewanella putrefaciens

Shewanella putrefaciens are found throughout the world in marine environments, and most reported human infections occur in countries with warm climates. Initially they were considered to be colonizers or saprophytes thriving on previously damaged tissue.^[14] *Shewanella putrefaciens*. Have

been implicated in skin and soft tissue infections. It is typically only seen to show effects in combination either other bacterial infection such as *E. coli*, *pneumonia*, and *streptococcus*. Soft tissue infections have various clinical manifestations including infected leg ulcer, cellulitis, abscess formation, and wound infection, which are often preceded by chronic ulceration of the lower limb, trauma, burn wound and sea water exposure^[15-17].

Salmonella spp.

Most infections are spread to people through consumption of contaminated food (usually meat, poultry, eggs, or milk). *Salmonella* infections affect the intestines and cause vomiting, fever, and cramping, which usually clear up without medical treatment. We can help prevent *Salmonella* infections by maintaining good hygiene around our self. Hand washing is a powerful way to guard against *Salmonella* infections. Salmonella outbreaks that occurred from 1985 to 1999, five hundred twenty two (62%) outbreaks of *S. enteritidis* infection were associated with food prepared at commercial food establishments (restaurants, caterers, delicatessens, bakeries, cafeteria, or market)^[18]. So, it's necessary to wash our hands, particularly after trips to the bathroom and before handling food in any way.

Bacillus cereus

Bacillus organisms are widely distributed in the environment although the primary habitat is the soil. These organisms are usually found in decaying organic matter, dust, vegetable, water, and some species are part of the normal flora. In the hospital setting, outbreaks and pseudo epidemic have been traced to contaminated ventilator equipment, disinfectant (ethyl alcohol), hospital linen and dialysis equipment. Sources of *B. cereus* in food borne outbreaks have been described including rice, meat loaf, turkey loaf, mashed potatoes, beef stew, apples and hot chocolate sold in vending machines. The clinical spectrum of infections caused by *Bacillus spp.* include self-limited food poisoning, localized infections related to trauma (e.g. ocular infections), deep seated soft tissue infections, and systemic infections (e.g. meningitis, endocarditis, osteomyelitis, and bacteremia

Method

Procedure followed:

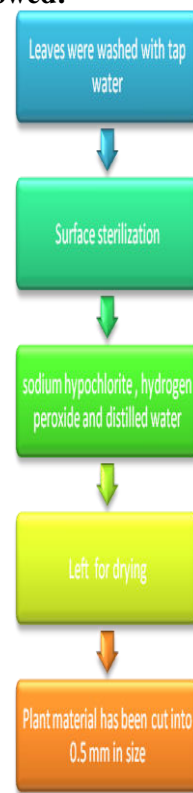


Figure1: Flowchart of the procedure followed for the sterilization of plant material

Surface sterilization

S. NO	Chemicals used	Concentration	Time (min)
1	Sodium hypochlorite	5%	5-10
2	Hydrogen peroxide	3-12%	5-10
3	Ethyl alcohol	70%	2

Table no. 2 chemicals used for the surface sterilization of plants with concentration and time of exposure.

Preparation of Plant extracts

Plant material was surface sterilized and left freely for drying. Then the plant material was coarsely powdered. 10 grams of each plant material was weighted and putted separately in solvents.

Plant species	Solvent used	Extraction process
<i>Azadirachta indica</i>	Methanol	Crude drug extraction
<i>Ocimum sanctum</i>	Ethyl alcohol	Crude drug extraction
<i>Cymbopogon</i>	Methanol	Crude drug extraction
<i>Allium sativum</i>	Ethyl alcohol	Crude drug extraction
<i>Cinnamomum camphora</i>	Methanol	Maceration Process
<i>Terminalia arjuna</i>	Ethyl alcohol	Maceration Process

Table no. 3 solvents and the extraction process method used for individual plant species.

Crude Drug Extraction Process

10 g of the powdered leaves of each plant were extracted with 100 ml of solvent solution (9 parts of solvent and 1 part of distilled water) by means of extraction. This mixture was heated at 60°C for 60 minutes. The content was filtered through Whatman filter paper in order to get particle free extract^[19]. Filtrate was used as solvent extract. From this method *Azadirachta indica*, *Cymbopogon* used methanol as solvent, while in *Ocimum sanctum* and *Allium sativum* methyl alcohol was used as the solvent.

The condenser is located at the top of the distillation column and removes energy from the distillation column. The purpose of the condenser is to condense the vapour leaving the top tray of the column thus making it cool. The purpose of water-cooled condenser in the extraction assembly is to convert gas from the substance into liquid. If the temperature and boiling point are kept constant, then the substance with lower boiling point will be able to evaporate and condensed, thus separated. The condenser ultimately just cools the gas form of the substance so it may return to the liquid state. It should also be noted that water should enter the condenser at the lower end because the apparatus is more efficient when

it is operated in that way. The difference in temperature between the cooling water and the substance to be distillate is what derives heat exchange. But we need temperature of difference (temperature gradient) between the cooling water and the distillate all the way along the condenser.

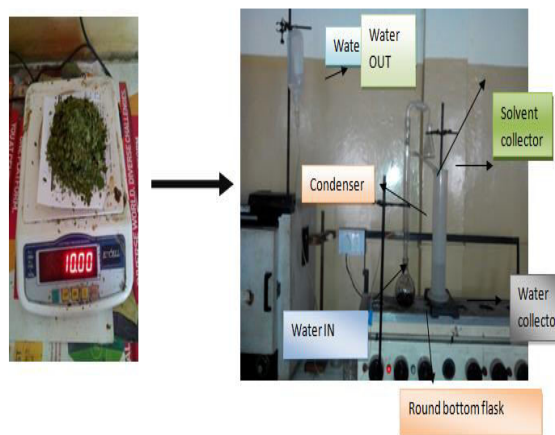


Figure 2: Plant extraction process by crude drug extraction

Use of methanol as solvent

Bioactive compounds from Plants belong to various chemical groups such as tannins, alkaloids, glycosides, lignans, terpenoids, etc. Methanol has a polarity index of 5.1. Mostly methanol is used for extraction various polar compounds but certain group of non-polar compounds are fairly soluble in methanol if not readily soluble. Therefore, methanol is commonly used for extraction of bioactive compounds. But if these compounds are strictly hydrophobic then either a mixture of methanol and chloroform for chloroform alone is used for extraction of bioactive compounds. Moreover, methanol among all the alcohols has low boiling point of just 65°C. So, extraction and concentration of bioactive compounds is easy.

Use of ethyl alcohol as solvent

Ethanol is less volatile and less toxic. Many secondary metabolites of plants are soluble in these solvents (which on their part also are neither too toxic nor too volatile for the manipulator) and can be partially purified. Ethanol can dissolve both polar and non-polar substances. So, it becomes second good choice for the extraction.

Maceration Process

Principle: In this process solid ingredients are placed in the Stopped container with the whole of the solvent and allowed to stand for at least 3 days (4-5 days) with frequent agitation, until soluble matter is dissolved. The mixture is then strained through sieves/net/filter paper, the marc pressed and the combine liquid is clarified by the process of filtration, after standing^[20].

Preparation of herbal hand sanitizer

Plant extracts	Quantity in ml
<i>Azadirachta indica</i>	2
<i>Ocimum sanctum</i>	2
<i>Cinnamomum camphora</i>	2
<i>Terminalia arjuna</i>	2
Glycerine	1
Distilled water	1

Table no. 4 Formulation of herbal hand sanitizer with quantity in ml.

Vitamin E was added as the preservative. Fragrance was also added. The total quantity made is of 10 ml. The composition of herbal sanitizer is based on the individual testing of each extraction on the *Bacillus cereus* plate, *Shewanella putrificins* plate and *Shalmonella spp.* plate. The highest zone of individual plant extract was noted and taken for the formulation of herbal sanitizer.

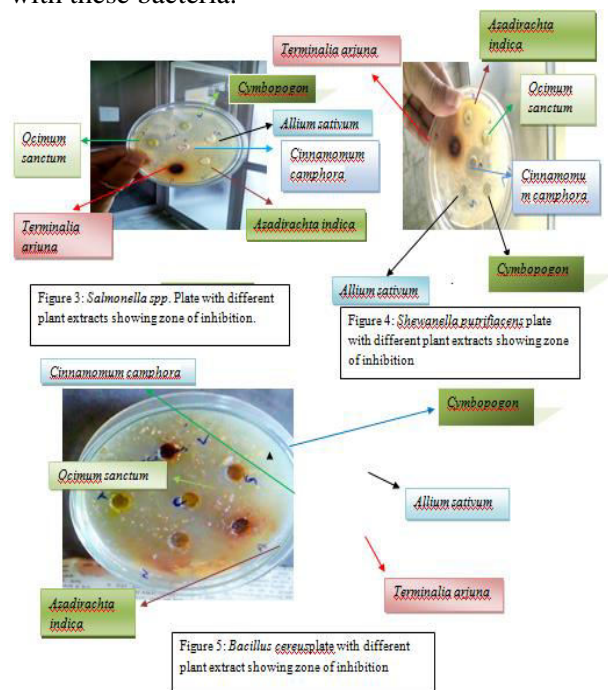
Antimicrobial screening

The screening of antibacterial activity of the extracts against pathogens was performed using dip well method. Nutrient agar media was prepared. Three sterile petri plates were taken for testing the anti-microbial activity against three different microorganisms i.e. *Shewanella putrificens*, *Salmonella spp.*, *Bacillus cereus*. The plates were filled with nutrient agar solution and allowed for solidification. After solidification the microorganisms from the subculture were inoculated into the nutrient agar media and six cavities were made in it. The cavities were filled with *Azadirachta indica*, *Ocimum sanctum*, *Cymbopogon*, *Cinnamomum camphora*, *Terminalia arjuna*, *Allium sativum* extracts. 0.5 ml of each extract was filled in these cavities. The plates are placed in incubator at 37° C to test the activity. After 24 hours the plates were observed for the formation of zone of inhibition^[21]. From the zone of inhibition, the anti-microbial activity of formulation is estimated.

Finger Impression Method: -Finger impressions were taken on the separate media plates. For this purpose, 4 nutrient agar media plates were made aseptically. Volunteers were asked to apply their finger impressions on it. On the first media plate finger impressions were taken without applying anything while on the second plate ordinary soap hand washing was done prior to finger impression. Then on the third plate herbal hand sanitizer was applied in the hands of volunteer before finger impression and in the last plate alcohol based herbal sanitizer was used by the volunteer and then impressions were taken. Then, these 4 media plates were incubated at 37°c for 18 hours. Visible colonies were formed, these colonies were directly counted by colony counter.

Results of both the methods used

Dip-well method: The antimicrobial activity of the individual plant extract was tested against *Shewanella putrificens*, *Salmonella spp.*, *Bacillus cereus* and the zone of inhibition were noted. The result from dip well method showed that *Terminalia arjuna*, *Azadirachta indica*, *Cinnamomum camphora*, *Ocimum sanctum* had shown good results with all 3 bacteria. So, in the formulation of herbal sanitizer these are used as the ingredients. While the *Allium sativum*, *cymbopogon* had shown no good results with these bacteria.



Plant extracts	Zone of inhibition in mm (<i>Salmonella</i>)	Zone of inhibition in mm (<i>Bacillus cereus</i>)	Zone of inhibition in mm (<i>Shwenella putrifaciens</i>)
<i>Azadirachta indica</i>	15	12	16
<i>Ocimum Sanctum</i>	17	14	10
<i>Cymbopogon</i>	11	10	12
<i>Cinnamomum camphora</i>	15	12	20
<i>Terminalia arjuna</i>	25	27	26
<i>Allium sativum</i>	8	9	9

Table no. 5 zone of inhibition in (*Salmonella*, *Bacillus cereus*, *Shwenella putrifaciens*) plates with the individual plant extracts.

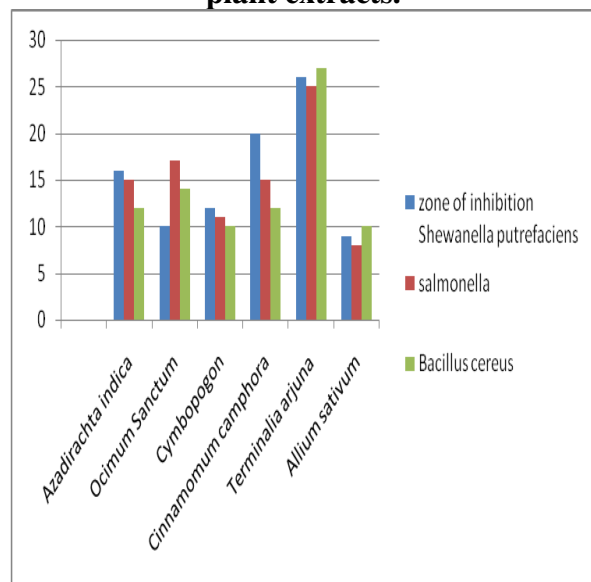


Figure 6: Graph showing zone of inhibitions with individual bacterial species taken in the study. It has been found that largest zone of inhibition was formed with *Terminalia arjunain* all 3 cases.

Finger impression method results:

Media plate no.	Material applied on hands	CFU
1	Nothing applied	395
2	Ordinary soap	280
3	Herbal sanitizer	150
4	Alcohol based herbal sanitizer	100

Table no. 6 Comparative study between free hand, ordinary soap, herbal sanitizer and alcohol based formulated herbal sanitizer impression counted directly with CFU.

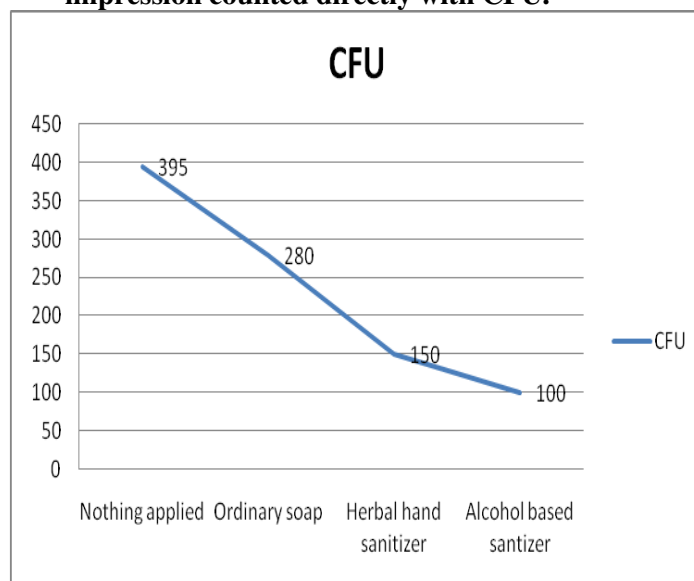


Figure 7: Graph showing the efficiency of herbal sanitizer by using colony counter. It has been shown that the remarkable number of colonies decreases by using herbal hand sanitizer, but alcohol based herbal sanitizer has proven best.

Conclusion

When it comes to *Azadirachta indica* it is reported to contain diterpenoids like nimbinone and nimbine which possess antibacterial activity against various gram positive and gram-negative organisms [22]. Hence a new way can be found using antibiotic resistance of pathogenic organisms and provide safe and healthier living through germ free hands, although the removal is not 100% but a major number of microorganisms can be reduced with natural herb, which is economical and safe [23]. From the above result of Dip well Method, it is cleared that the herbal hand sanitizer should be formulated with these plant

extracts ie: *Terminalia arjuna*, *Ocimum Sanctum*, *Cinnamomum camphora* and *Azadirachta indica*. When these plants will combine their combination can give enough good inhibition against variety of pathogens. Use of herbal compounds in making antiseptic lotions in place of chemicals available hand sanitizers might be good for people whose skin is sensitive with chemicals. It is a relief for those people who possess the skin allergic problems like the formation of burst, redness, white patches, inflammation etc, seen on their skins, so herbal hand sanitizers are natural, cheap, and safe [23].

Physical Parameter Results

Herbal sanitizer test	Results
Colour	Dark red
pH	6.81
Odour	Sweet lemon
Stability	No change
Skin irritation	No

Table no. 7 Results of physical parameters of herbal sanitizer.

From the results of Finger impression method, it is well suggested that the combined plant extracts are well capable to retard the bacterial colonies. Use of herbs in preparation of herbal hand sanitizer and use of these compounds in making antiseptic lotions in place of chemicals available hand sanitizers might be good for the people whose skin is sensitive with chemicals. An appropriate quantity of alcohol should also be used for the formulation as it also possesses best antimicrobial activity.

In many sanitizers there are many harsh chemicals, which have really dangerous impact on human's so the alternate of this is herbal sanitizer as because of many advantages over the chemical sanitizer.

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