



International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

The Creation and Assessment of a Herbal Sanitizer Utilizing Natural Herb Genus Helianthus

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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, Cymbopogon, Cinnamomum camphora, Terminalia arjunawas 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 handsanitizer 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. Genus helianthus Will Reduce the dryness of skin after rubbing the sanitizer on hand. Also works for betterment of skin and bone health.

Keywords: Herbal Sanitizer, Antibacterial, Antimicrobial, Genus Helianthus

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. 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. 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.

The US Centers for Disease Control and Prevention, along with numerous other international governmental authorities, suggest hand sanitizers as a suitable substitute for soap and water in terms of personal hygiene. There are numerous sanitizers on the market, and many of them include dangerous compounds that can irritate skin and make germs resistant. Health care workers' hands are the main way that these multidrug-resistant pathogens and infections are spread to patients. The most important, straightforward, and least expensive way to prevent infections linked to healthcare and the spread of antibiotic resistance is to practice good hand hygiene. Unfortunately, poor hand hygiene practices are seen because of a lack of scientific knowledge, ignorance of the risks, misconceptions (such as the idea that wearing gloves negates the need for hand hygiene), a lack of hand hygiene facilities, understaffing, and patient overcrowding.

Numerous skin infections are caused by organisms such as Salmonella species, Bacillus subtilis, E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans. In the past, plants have been a good supply of infectious agents for ants. An enormous unexplored medical resource is plant-based antimicrobials.

According to one study, simple practices like washing your hands can lower the rate of blood flow connected to catheterization. There are many sanitizers available in the market and they contain many harmful chemicals which may cause skin irritation and also resistant among pathogens. 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. 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. 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-kampferol, elimicin, catechol, 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 antifungal 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. Keeping in view the ultradisinfectant 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. 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 saffrole. 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. 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%.

Genus *Helianthus*

The genus *Helianthus*, commonly known as sunflowers, offers several medicinal benefits, particularly its seeds and oil. These benefits include antioxidant, anti-inflammatory, and cardiovascular support, along with potential benefits for skin and bone health. The sunflower plant contains several chemicals in its various organs, such as alkaloids and phenolics in the leaves, saponin in the flower parts, polyphenol in the root part containing alkaloids, and fatty acids and tannins in the seeds. Several studies have shown that the *Helianthus annuus* extracts by organic solvents have potential antimicrobial effects against different Gram (+) and Gram (–) bacterial strains. The antimicrobial activity of methanolic extract of seeds from *Helianthus annuus* was studied against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholera*. The seed extract of *Helianthus annuus* showed high activity against *Salmonella typhi*, moderate activity against *Staphylococcus aureus* and *Vibrio cholera*, and less activity against *Bacillus subtilis*. The studies by provide evidence that the properties of sunflower seeds are known to inhibit the growth of bacteria in water. Therefore, the present study aimed to evaluate the antimicrobial properties of sunflower seeds and their effectiveness in inhibiting the growth of selected bacterial sub-divisions, notably faecal coliforms, total coliforms, and *E. coli*. The sunflower plant contains several chemicals in its various organs, such as alkaloids and phenolics in the leaves, saponin in the flower parts, polyphenol in the root part containing alkaloids, and fatty acids and tannins in the seeds. Several studies have shown that the *Helianthus annuus* extracts by organic solvents have potential antimicrobial effects against different Gram (+) and Gram (–) bacterial strains. The antimicrobial activity of methanolic extract of seeds from *Helianthus annuus* was studied against *Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Vibrio cholera*. The seed extract of *Helianthus annuus* showed high activity against *Salmonella typhi*, moderate activity against *Staphylococcus aureus* and *Vibrio cholera*, and less activity against *Bacillus subtilis*. The studies by provide evidence that the properties of sunflower seeds are known to inhibit the growth of bacteria in water. Therefore, the present study aimed to evaluate the antimicrobial properties of sunflower seeds and their effectiveness in inhibiting the growth of selected bacterial sub-divisions, notably faecal coliforms, total coliforms, and *E. coli*.

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>Terminalia arjuna</i>	Bark
<i>Cinnamomum camphora</i>	Leaves
<i>Genus Helianthus</i>	Leaves

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. *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. *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) . 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:

Step1:- Leaves were Washed with tap water

Step2:- Surface Sterilization

Step3:- Addition of Sodium Hydrochloride and distilled water

Step4:- Left for Drying

Step5:- Plant Materials Are cut in to 0.5 min. size

Surface sterilization

S.no.	Chemical Used	Concentration	Time (min)
1	Sodium Hydrochloride	5%	5-10
2	H ₂ O ₂	3-12%	5-10
3	C ₂ H ₅	70%	2

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

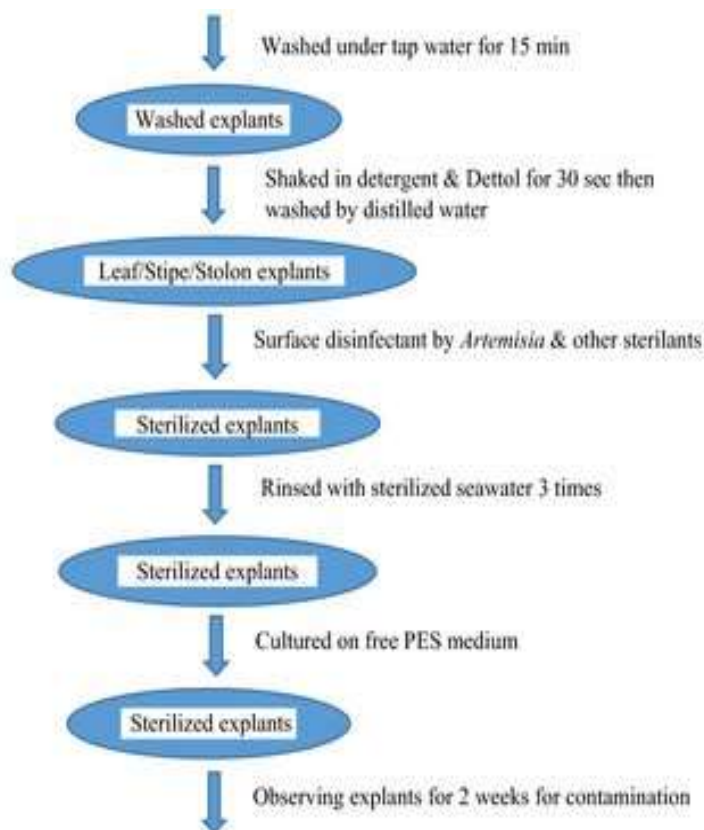


Figure1: Flowchart of the procedure followed for the sterilization of plant material

Plant Extract Preparation :-

After being surface sterilized, the plant material was allowed to air dry. The plant material was then ground into a coarse powder. Ten grams of each plant item were weighed and added to solvent separately.

Plant Species	Solvents Used	Extraction Process
Azadirachta Indica	Methanol	Crude Drug Extraction
Helianthus annuus L.	Ethyl Alcohol	Crude Drug Extraction
cymbopogon	Ethyl Alcohol	Crude Drug Extraction
Allium sativum	Ethyl Alcohol	Maceration Process
Cinnamomum camphora	Methanol	Maceration Process
genus Helianthus	Methanol	Maceration Process

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

Method for Crude Drug Extraction Using 100 milliliters of solvent solution (one part distilled water and nine parts solvent), 10 grams of each plant's powdered leaves were extracted. For 60 minutes, this combination was heated to 60°C. To obtain a particle-free extract, the material was filtered using Whatman filter paper. As a solvent extract, the filtrate was employed. Methanol was utilized as the solvent in Azadirachta indica and Cymbopogon, whilst methyl alcohol was utilized in Ocimum sanctum, Allium sativum and genus Helianthus.

Energy is extracted from the distillation column by the condenser, which is situated at the top of the column. The condenser's job is to cool the column by condensing the vapor that exits the top tray. The extraction assembly's water-cooled condenser's job is to turn the substance's gas into liquid. The substance with a lower boiling point will be able to evaporate and condense, allowing for separation, provided that the temperature and boiling point remain constant. In the end, the condenser merely cools the substance's gas form so that it can transform back into a liquid.

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 Stoppered 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.

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 petrificans* plate and *Shalmonella* spp. plate. The highest zone of individual plant extract was noted and taken for the formulation of herbal sanitizer.

Preparation of herbal hand sanitizer

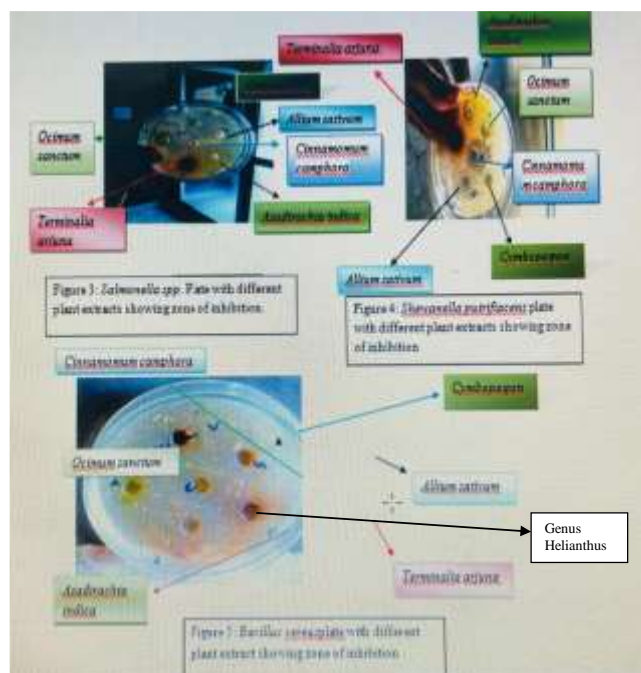
Sn.	Plant Extract	Quantity In ml.
1	<i>Azadirachta Indica</i>	2
2	<i>Helianthus annuus L.</i>	2
3	<i>cymbopogon</i>	2
4	<i>Allium sativum</i>	2
5	<i>Cinnamomum camphora</i>	2
6	Glycerine	1
7	Distilled Water	1

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 putrifaciens*, *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 putrifaciens*, *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*, *Helianthus annuus L.* 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 Plant Extract	Zone of inhibition in mm (salmonella)	Zone Of Inhibition In mm.(Bacillus Cerus)	Zone Of Inhibition mm(Shwnella putrifacie)
Azadirachta Indbia	15	12	16
Helianthus annuus L.	17	14	10
cymbopogon	11	10	12
Allium sativum	15	12	20
Cinnamomum camphora	25	27	26
genus Helianthus	25	27	26

Conclusion

Flavonides including nimbinone and nimbine, which have antibacterial properties against a variety of grampositive and gramnegative pathogens, are said to be present in Azadirachta indica. Therefore, a new method that uses antibiotic resistance and skin and bone health of harmful organisms can be found to give safe and healthy living through germfree hands.

While the elimination of all microorganisms is not 100%, a significant percentage of them can

be reduced with natural herbs, which is safe and costeffective. It is evident from the Dip Well

Method's above result that these plants should be used to manufacture the herbal hand sanitizer.

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