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FORMULATION AND EVALUATION OF POTENTIAL ANTIMICROBIAL ACTIVITY OF POLY HERBAL HAND WASH.

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Abstract:
The current work's objective was to create formulations of herbal handwash using Azadirachta indica (Neem) leaf, orange peel, and aloe vera gel extracts. The hand wash formulation was created, and its physical characteristics, including appearance, pH, and viscosity. Hand wash compositions were tested for antibacterial efficacy against skin infections. The findings showed that the herbal hand wash formulation had a sizable zone of inhibition.compared to the usual medication (powdered Neosporin). Therefore, these plant components can be used to make herbal hand soap on a large scale.
Key Words: Polyherbal handwash, Antimicrobial Activity, Neem, Orange peel.

1. Introduction

Hand hygiene is the most effective approach to stop the spread of dangerous germs and disease because hands are the main method of microbe and disease transmission. Hand washing is a crucial precaution to take in order to stop the transmission of infectious diseases.[1]

The major goal of hand washing is to remove filth, harmful germs, soil while preventing the spread of transitory microbes. Hygiene techniques can stop the viral or bacterial spread of infection. As a result, it raises the concern of hygiene hand washing. There are now several antimicrobial substances available in alcohol-based hand soap, detergent, and other products. Notwithstanding some drawback or unfavorable reaction, these soaps or solutions can avoid microbial contamination in the healthcare setting.[2],[3]

Only a few reports exist on the inhibitory activity against specific pathogenic bacteria and fungi. Some indian medicinal plants were reported to have antimicrobial properties based on folklore information. For treating and curing a variety of medicinal applications, including wound healing and inflammation treatment. Plants have been a reliable source of antimicrobial activity, and plants extracts have the potential to act as antimicrobial agents against a variety of pathogenic microorganisms that cause infection, disease, and medication resistance. The fundamental benefit of choosing natural source is that they are cheaper, more readily available, and safer than chemicals.[4],[5]

1.1 Benefits of polyherbal hand wash

- Easy to use: Herbs are easily accessible both in urban and rural areas, making theiruse simple for everyone.
- Affordable: The cost of herbal plants is lower than the cost of the chemicalingredients in synthetic hand washes.
- Enhanced Efficiency: The promotion of good hand hygiene is more effective with herbal hand washes.
- Less Negative Impact: Herbal hand washes are less likely to have side effects than other hand washes.
- Additionally, it aids in the resolution of fungal and antiseptic skin issues.
- It also helps in efficient removal of oil and impurities from skin.
- More convenient than using soap and water.
- The simplest method of eliminating microorganisms.
- Washing your hands helps keep germs out of your body.[6]

1.2 Advantages And Disadvantages

Polyherbal handwash refers to a hand sanitizer or handwash that contains a combination of multiple herbal extracts. Here are some potential advantages and disadvantages of using polyherbal handwash:

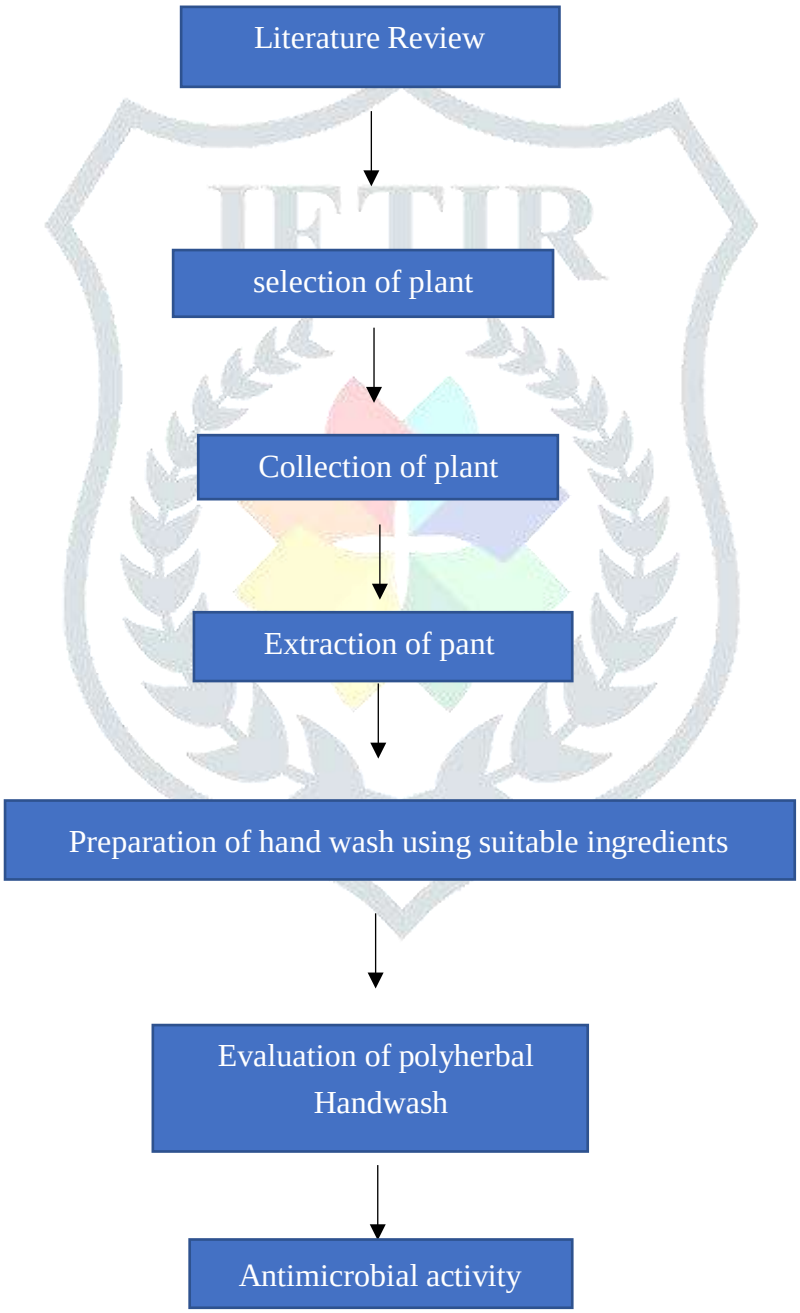
Advantages:

- Natural ingredients: Polyherbal handwash is made from natural ingredients, which means it is free from harsh chemicals that may cause skin irritation or other adverse effects.
- Antimicrobial properties: Many herbs used in polyherbal handwash have been traditionally used for their antimicrobial properties, which can help to kill harmful bacteria and viruses on the skin.
- Variety of benefits: Because polyherbal handwash contains a combination of herbs, it may offer a broader range of benefits than a single-ingredient handwash.

Disadvantages:

- Limited research: There is limited research available on the effectiveness of polyherbal handwash compared to other types of hand sanitizers or handwashes.
- Varying effectiveness: The effectiveness of polyherbal handwash can vary depending on the quality and concentration of the herbal extracts used, which can be difficult to regulate.
- Potential for allergic reactions: Some people may be allergic to one or more of the herbs used in polyherbal handwash, which could cause skin irritation or other adverse reactions. [7],[6]

2. Plan of Work:



3. Materials and methods:-

3.1 Materials:-

Leaves of Neem, Aloe Vera gel, Orange peel, Glycerine, Methylparaeben, Sodium Lauriyal Sulphate (SLS), carbapol 940, Lemon Grass Oil.

5.1.1 NEEM:-

In Indian subcontinent, the Neem tree (Azadirachta indica) has long been referred to as the "wonder tree''since it has so many different medicinal applications. Neem has grown in significance in today's worldwide setting. Neem leaves have amino acids, saponins, flavanoids alkaloids, tannin's, and saponoids Neem and its extracts are found in many allopathic and natural medications. Dermatitis, eczema ,acne, bacterial, fungal infection, and other skin conditions are treated with neem. Neem has historically been used to treat ailments that purify the blood and skin, Neem not only aids in the treatment of illness, but it also boosts our immune, giving us the fortitude to fend off illness.

Taxonomical description of neem:-

Kingdom : Plantae

Division : Magnoleophyta

Order : Sapindales

family : Meliaceae

Genus : Azadirachta

Species : A. Indica

Fig 1. Neem leaves.

Uses:-

- Providing relief for skin condition including psoriasis and eczema.
- Treating dandruff, itching, and other ailments of the scalp.
- Healing the wounds.
- Treating and curing ringworm, burns, infected wounds, and fungus infections treatingathlete's injuries.
- There are a numerous medical preparations made from all parts of the Neem tree. [8],[9]

5.1.2 ORANGE PEEL :-

Oranges are also high in calcium, folic acid, potassium, beta-carotene, amino acids, pectin, and fibre. They are also high in iron, chlorine, manganese, zinc, salt, salt, phosphorus, iodine, and sodium. One oranges contains about 60 flavonoid with anti-tumor, anti-inflammatory, blood clot-inhibiting, and antioxidant effects, as well as about 170 phytonutrients. Most citrus fruit is used in industry, but the peels are typically thrown away. Appropriate techniques must be used to convert orange peel and pulp into a value-added product. Its also possible to lessen environmental contamination. Orange peels are a key dietary source of the antioxidant phenolic. The pee of oranges are nutrient-rich. It has a lot of phytochemicals. They are helpful because of this. They can be utilized in a variety of foods and medications. Fiknding the right application for these peel is crucial. Athe orange peel that is generates during the

preparation of orange juices and other orange relates goods builds up in large quantities and cause environmental issues. Orange peel provide three to four times as much fibre and a flavonoid as a whole fruit. They are advantageous for heart health. Oranges peel contains a flavonoid called hesperidin, which has anti-inflammatory properties.

Taxonomical description :-

Kingdom : plantae

Division : Mangoliophyta

Class : Mangolipsida

Order : Sapindales

Family : Rutaceae

Genus : Citrus

Fig 2. Orange Peel

Uses :-

- The Pulp of oranges were used to create cosmetics in other places.
- It is useful as a general tonic.
- It is used for the treatment of angina, Constipation's, Menstrual disorders, Hypertension.
- Detoxify the skin.
- It has Anti-microbial and anti-bacterial properties make it an excellent choice to treat oilyskin and mild acne. [10],[11]

5.1.3 ALOE VERA :-

Aloe Vera is a succulent plant that thrives in desert and subtropical areas. It is utilized in alyurvedic, Homeopathic and allopathic system of medicine for its therapeutic benefits. Aloe vera has been utilized for its healing and therapeutic properties for a long time. Although more than 75 active components from the inner gel have been found, the therapeutic effects of each particular chemical have not been adequately connected, However, it is thought that these biological activities should be attributes to a synergistic action of the compound contained therein rather than a single chemical substance. Many of the medicinal effects have been attributed to the polysaccharides found in the inner leaf paernchymatous tissue. The processing of the leaf pulp has grown into a significant global industry. Aloe vera is most commercially successful aloe species. In the food industry.

Taxonomical description :-

Kingdom : Plantae
Order : Asparagales
Family : Asphodelaceae
Genus : Aloe
Species : A. Vera

Fig 3. Aloe Vera

Uses:-

- Healing Wounds.
- Anti-inflammatory action and immunity activity.
- It is an excellent moisturizer for skin.
- Antioxidant activities.
- Antiviral and anti tumor activities.
- Used in cosmetics.[12]

3.2 Methods:-

5.2.1 Collection of plant material:-

All the herbal plants like Neem, Aloe Vera, Orange peel are collected from thelocal places, Abhona(Maharashtra).

5.2.2 Preparation of herbal plant extract:-

5.2.2.1 Extraction Of Azadirachta Indica:-

The fresh Neem leaves were collected and dried at room temperature. The dried leaves thenpowdered, Then powdered neem leaves are weighed 30grams and macerated in beaker using 250ml of ethanol. The prepared mixture is covered and kept 3 days for maceration while stirring in between, and then the mixture was filtered using filter paper. Then the filtrate werecollected for preparation of polyHerbal hand-wash. [13],[14]

Fig 4. Neem extract

5.2.2.2 Extraction of Orange peel:-

The orange peel was collected and dried at room temperature. The dried peel was powdered, then powdered orange peel are weighed 20 grams and macerated in beaker using 250ml of ethanol. The prepared mixture is covered and kept for 3 days for maceration while stirring in between and then the mixture was filtered using filter paper. Then the filtrate were collected for preparation of polyherbal hand wash. [15]

Fig 5. Orange peel extract

5.2.2.3 Gel extraction process from aloe vera pulp :-

Aloe Vera leaves were used to extract gel, which was done by scrapping away the mucilage and exudates with a blunt-edged knife to ensure uniformity the mucilage given through a mixin a blender. A muslin cloth was used to filter and strain the solution.[16]

Fig 6. Aloe Vera Extract

4. Formulation Table:

Sr. No.	Ingredients	Quantity (gm/ml)	Uses
1	Neem	12ml	Antimicrobial Agent.
2	Orange peel	8ml	Antibacterial Agent
3	Aloe vera gel	6ml	Healing Agent
4	SLS	4gm	Foaming Agent
5	Carbopol 940	2gm	Gelling Agent
6	Methyl Paraben	2gm	Preservative
7	LemonGrass Oil	4ml	Perfume
8	Glycerine	12ml	Moisturising Agent
9	Distilled Water	Q.S.	

Table No.1: Formulation table

5. Method of preparation :-

- Neem, Orange peel extract, and Aloe Vera gel measured accurately and dissolved by gentle heating.
- After heating, keep the solution aside for sometime.
- The required quantity of sodium lauryl sulphate dissolved in distilled water along with glycerin were mixed in above solution with continuous stirring.
- The methyl paraben was dissolved and mixed with the extract.
- Then the required quantity of lemon grass oil were added for fragrance.
- The required quantity of Carbopol 940 taken, and the above formulation was added while being continuously stirred with a mechanical stirrer to create a homogeneous gel.
- Finally, it was kept in container that was tightly locked and well labeled for further analysis.[17]

6. Evaluation of poly herbal hand-wash:

6.1 Organoleptic Evaluation: The color and texture were evaluated by visual and touch sensation respectively. The odour was inspected by sensing the formulation.

Colour : Dark Greenish.Odour : pleasant.
Texture : Smooth.

6.2 Appearance and Homogenicity: Appearance and Homogenicity was evaluated by visualInspection. [18]

6.3 Grittiness: 1ml of Gel was taken on finger tips and rubbed between two fingertips, then the formulation was evaluated. [21]

6.4 Skin irritation test: Skin Irritation Test was evaluated by applying Polyherbal Hand wash on skin and left for 30 min, after 30 minutes of washing observe any itching, rashes or redness on skin by sensory and visual inspection. [6],[19]

6.5 pH: 1gm of Sample of Polyherbal Hand wash Gel was taken and Dissolved it into 100ml distilled water. The pH solution was Measured by standardized digital meter.[22]

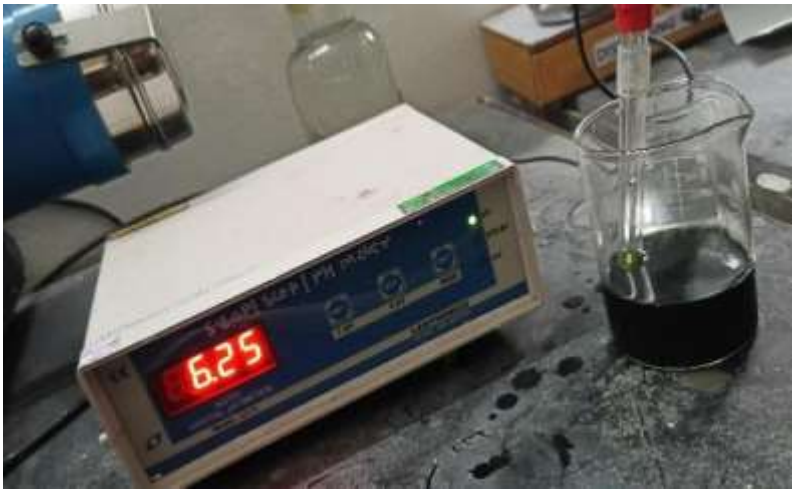


Fig 7. Ph Determination

6.6 Viscosity: The viscosity of Poly-herbal Hand wash Gel was determined by using Digitalviscometer. [20]

Fig 8. Viscosity at 3.0Rpm

Fig 9. Viscosity at 6.0Rpm

Fig 10. Viscosity at 12.0Rpm

6.7 Foam Height: 5 ml of poly-herbal hand wash was taken in a beaker. Then a small amount of water was poured into it from a small height. The height of the foam was measured. [20]

Fig 11. Foam Height.

6.8 Spreadability: 0.5 gm of sample of polyherbal hand wash was taken and passed between two slides and left for 5minute where no more spreading was expected diameter of spreader circle was measured in cm and was taken as comparative value for spread ability. [22]

6.9 Stability study: Stability study were performed on formulation by subjecting the product to temperatures of 24^0 C, 37^0 C & 45^0 C for 24 Hours. During the stability test, there was no color change or phase separation in a prepared handwash. [20]

7. Evaluation parameter teste:

Sr. No.	Evaluation parameters	Polyherbal Hand Wash
1)	Colour	Dark Green
2)	Odour	Pleasant
3)	Texture	Smooth
4)	Grittiness	Non-Girtty
5)	Appearance and Homogenecity	Translucent
6)	Skin irritation Test	Non irritant
7)	pH	6.25
8)	Viscosity	37mpa'S
9)	Foam Height	3.2cm
10)	Spreadability	Easily Spreadable
11)	Stability	Stable

Table No.2: Evaluation parameter teste

8. Antimicrobial Activity:

9.1 Inoculum:-

Start by preparing the inoculum. This involves selecting a bacterial strain to test the handwash against. Transfer a small amount of the bacteria from a pure culture onto a sterile nutrient agar plate. The plate is incubated overnight at the appropriate temperature, which shows the growth of bacteria.

Fig 12.Inoculate nutrient broth tube from Plate culture

9.2 Bacterial Culture

- Nutrient broth was prepared according to procedure. Which provides the necessary nutrients for Bacterial growth. Then, an appropriate amount of sterile nutrient broth is transferred into a sterile test tube. Using a sterile loop wire, transfer a small amount of bacteria from the overnight culture onto the nutritional broth. This process is called inoculation. Then, inoculated nutrient broth was placed for incubation at +-37°C for 24 hours.

Incubation for ±37°C for 24 HoursNutrient broth + Bacteria

Fig 13. Bacterial culture

9.3 Turbidity:

The intended bacterial growth can be seen as turbidity in the inoculated nutritional broth that has been placed for incubation. The bacterial density in the culture is measured by turbidity and can be seen visually.

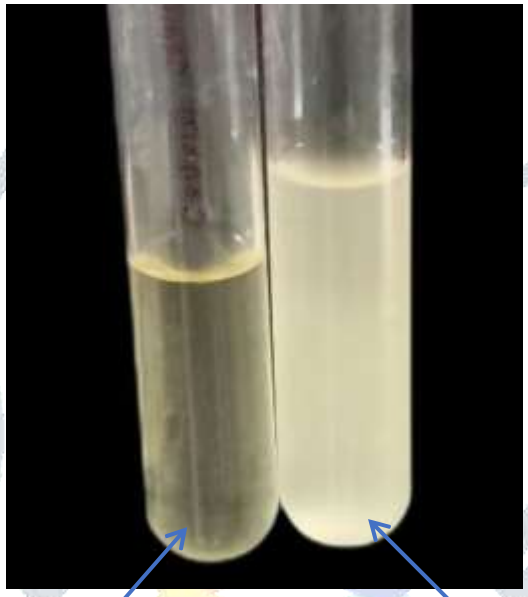


Fig 14. Turbidity

Control Sample

Bacteria Inoculum Sample
Turbidity form after inoculation

9.4 Lawn culture

- The sterile agar plates was prepared by pouring molten agar into a sterile petri dish andallow it to solidify.
- Using a sterile cotton swab or spreader, streak the surface of the agar plate with thebacterial culture obtained from the nutrient broth.

- Ensure the even distribution of bacteria across the entire plate creating a lawn of bacterialgrowth.

Fig 15.Lawn culture

9.5 Antimicrobial Disc:

- On the prepared lawn culture the polyherbal handwash can be tested for its antimicrobialactivity.
- Applied a specific amount of the handwash solution onto a one sterile filter paper discand neosporine powder solution onto a second sterile filter paper disc.
- Placed discs containing the one with hand wash and and another with neosporinesolution.
- The plates placed for incubaion at +-37°C temperature for a 24 Hours.

Fig 16. Antibcterial disc applied in agar plate

9.6 Observation:

After the incubation, observe the plate for any zone of inhibition around the disc. Thse zones around the handwash sample with respect to standard substance indicates antimicrobialactivity against the bacteria.

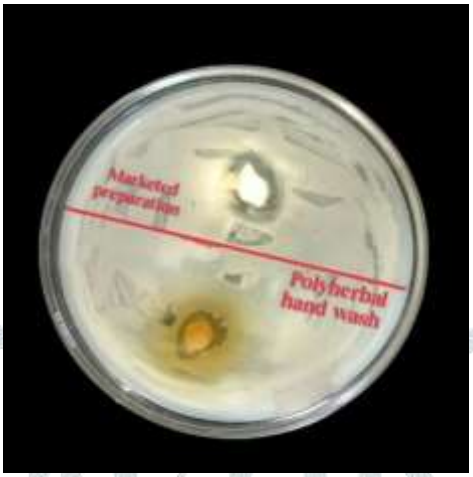


Fig 17. Zone of inhibition

9. Result and discussion:

Literature shows that neem leaves (Azadirachta indica) have antimicrobial properties, Orange peel extract they have an antibacterial effect, so this study was Designed to make a multi- herbal hand wash with antimicrobial and antibacterial properties the Handwash was dark green in color, non-greasy, smooth in texture and easy to wash with a good pH close to normal skin PH range No skin irritation was observed within a few days of use. Of all the studies we will eventually do notes that poly herbal hand wash has been proven to clean without irritating the skin and is easy to use because it is poly herbal. hand washing, thus reducing the possibility of side effects.

10. Conclusion:

Like cosmetics and cosmeceuticals, which are applied topically and purport to have medical effects have components that affect how skin behaves biologically. According to the WHO, the majority of people in Asian countries currently prepare their own hand wash using herbal medicine for hand hygiene. The goal of the current study was to create a polyherbal hand wash gel that contains herbal extract and can be used to prevent bacterial growth in addition to cleansing hands. Its ingredients were chosen based on how delicate the skin is, preventing any potential discomfort.Therefore, it can be said that polyherbal hand wash gels are far superior to basic soaps or already available hand washes because of their ingredients, effectiveness on our hands' skin, and suitability for all types of skin.

11. Future Prospects:

The market today offers many chemical hand washes as alcohol-based sanitizers made of different synthetic detergents. Alcohols and detergents do help to stop the spread of hazardous infections among healthcare workers, but they also have drawbacks and damaging effects on the environment and human tissues. The frequent use of such synthetic chemical-based formulations can cause pathogen resistance as well as skin discomfort. Due to the use of synthetic compounds and alcohols, the production costs of such synthetic formulations are also significant. In order to solve these issues, natural components must be used in place of synthetic chemicals. Natural substances are safe for use on both the environment and human skin. Thus, the development of herbal hand washing as a new method to combat pathogenic organisms that are resistant to antibiotics while also promoting safe, healthy, and natural living through the use of hands that are free of germs. As these herbs are readily available in the market, they can help lower the cost of manufacturing and have been shown to be more affordable than synthetic chemicals.

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