



# FORMULATION AND EVALUATION OF HERBAL AVOCADO FRUIT HANDWASH

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**Abstract:** Natural medicines are more accepted because they are safer and have fewer side effects than manufactured ones. On the global market, demand for herbal formulations is increasing. The present work deals with the development & evaluation of the herbal hand wash containing methanolic extract (9 parts methanol: 1 part water) of Avocado Fruit (*Persea Americana*). Hand washing is an essential part of daily living. Because hands are a primary source of microbial illnesses, proper hand washing with appropriate hand wash formulation is necessary. Herbs are recognised to have antibacterial qualities, thus using them as an antimicrobial agent is now widespread practice. Present study involves formulation of herbal hand wash using extract of Avocado Fruit. Agar Well diffusion method was utilized for evaluation of the antimicrobial activity against skin pathogens of the prepared herbal hand. Its effectiveness was evaluated and compared to that of a regular commercial hand wash. Results revealed that extract of Avocado Fruit formulation was more efficient in reducing the number of organisms from hands based handwash with less or no side effects. To avoid the side effect of synthetic handwash like itching, dryness and burning, an attempt has been made to develop a herbal handwash. It gives anti-microbial, anti-oxidant, anti-inflammatory properties and provide moisture to the skin. Thus, owing to higher efficacy these herbal extract can be used in the preparation of herbal hand wash on commercial scale.

**Keywords - Avocado (*Persea Americana*), Phytochemical Screening, Antimicrobials, Agar Well Diffusion Method.**

## INTRODUCTION

Medicinal plants are of great importance in the field of treatment and cure of disease. Scientific study has increased our understanding of the chemical processes and make-up of the active ingredients that give plants their therapeutic capabilities over time. Now it has become a universally accepted fact that Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones.

Hippocrates (400–370 BC) is the father of medicine. Hippocrates, a Greek philosopher and physician, simply believed in the fact that superstition ruled the society of that period. He applied reason and logic to practical medicine.

Herbal medicine is also known as botanical treatment or phyto-medicine which refers to the use of seeds, roots, leaves, bark, flowers and aerial parts of any plant for medicinal purpose. The demand for herbal formulations continues to increase on the global market. Herbal remedies have been the cornerstone of disease prevention and treatment in India since the dawn of civilization. Physiological conditions practiced in traditional methods such as Ayurveda, Unani and Siddha (Burke, 2003). Herbal medicines with many medicinal uses such as wound healing, treating inflammation Due to infection, skin sores, leprosy, diarrhoea, scabies, venereal diseases, snake bites and ulcers etc.

Hand cleanliness is the most crucial preventative strategy to stop the spread of dangerous germs and diseases because hands are the primary means of transmission for bacteria and infections<sup>1</sup>. The WHO recommends that everyone wash their hands before starting and concluding food preparation, eating, attending to a sick person, tending to a cut or wound, using the restroom, changing diapers, and cleaning up a child who has used the restroom, as well as before and after all of these activities. Following nose-blowing, coughing, or sneezing, after handling pet food or treats, after touching animal excrement, and after touching garbage<sup>2</sup>.

Herbal Hand wash Preparation is related to hygiene practices to reducing or preventing disease and spread of disease. It is mainly used because it is free from chemicals and all ingredients are obtained naturally. Hand washing is an important way to help fight the spread of disease. Hand washing helps to lower the amount of dangerous bacteria on hands and removes visible dirt.

## Hygiene

It is essentially characterised as a field of science concerned with information and practises related to health promotion<sup>3</sup>. Following cleanliness practises can help to avoid the spread of diseases (both bacterial and viral). Cleaning techniques (e.g., hand washing, bathing) are often employed to achieve hygiene because they remove dirt and soil as well as infectious germs<sup>4</sup>. Hygiene is described as the practise of maintaining cleanliness, which is of highest significance in maintaining health. The use of cleansers and maintaining personal cleanliness are necessary for healthy life. These ideas emphasize the significance of maintaining hygiene in illness prevention<sup>5</sup>.

## Hand Hygiene

Generally, hand hygiene refers to the practice of washing hands with water, soap, or another liquid<sup>6</sup>. The benefit of washing hands is that it removes pathogens (bacteria and viruses) and toxic substances<sup>7</sup>. Hand hygiene is particularly crucial for persons who work in the medical industry, restaurants, or cook and serve food to the general public<sup>8</sup>. Hand hygiene has been shown to reduce the spread of cold viruses and other germs. Washing one's hands is widely regarded as the most effective approach to maintain personal hygiene and protect oneself against disease<sup>9</sup>.

## Hand Washing

The skin is one of the most exposed parts of the body and must be protected from viruses. Hand washing is an essential precaution for protecting the skin from harmful microorganisms and preventing the spread of many contagious diseases. The best technique to ensure the eradication of transient bacteria is to wash hands and fingertips using a fingernail brush<sup>10</sup>. It means using either ordinary or antimicrobial soap or water to wash one's hands<sup>11</sup>.

Hand washing helps to lower the amount of dangerous bacteria on hands and removes visible dirt. Salmonella and E. coli are two harmful bacteria and viruses that can be carried by people, animals, or equipment and transferred to food<sup>12</sup>. In actuality, it can range greatly from a quick hand rinse to a thorough scrub. In a medical context, washing hands is done to get rid of pathogenic microorganisms (germs) and prevent their spread<sup>13</sup>.

## Effective hand washing requires six steps<sup>14</sup>

Step 1: Apply soap to wet hands. Rub palms collectively until soap starts to bubble.

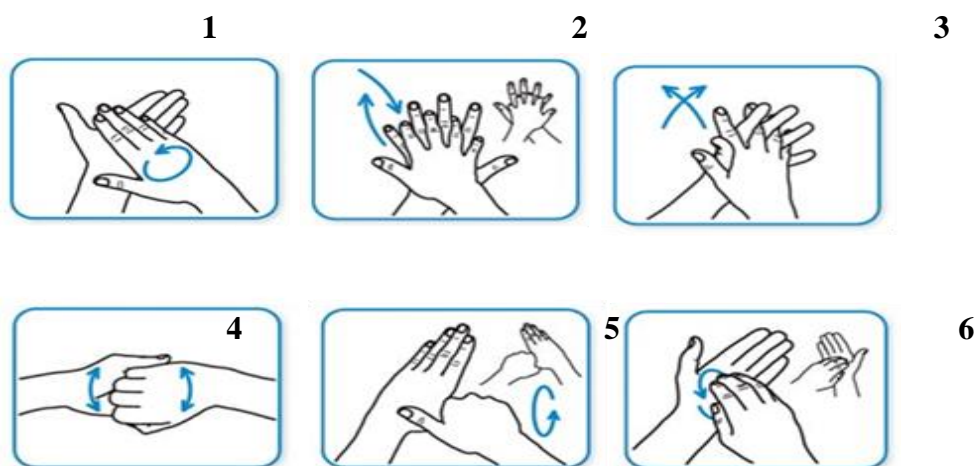
Step 2: Gently rub the back of the opposite hand with each palm.

Step 3: On each hand, rub in between your fingers.

Step 4: Rub your hands together, using all of the fingers.

Step 5: Rub your thumbs together.

Step 6: Rub your palms in circular motions. Next, rinse and pat your hands dry.



**Fig. 1: Steps of Hand Washing**

### **Herbal Hand wash**

Herbal hand soap is primarily utilized because, unlike regular hand soap, it is chemical-free. All of the herbal hand soap's ingredients are natural and secure. The use of herbal hand washes helps stop the transmission of germs and viruses since they are very gentle but very efficient. While using herbal hand wash, the mild foaming action does not irritate. It also aids in efficiently cleansing the skin of oil and grime. Additionally, it aids in the resolution of fungal and antiseptic skin issues<sup>15</sup>.

### **Herbal Plant**

The evergreen avocado tree, *Persea Americana*, is most frequently found in central and northern South America and belongs to the Lauraceae family. The avocado tree stays green year-round. Its height is between 40 and 80 feet above the ground, and it has numerous lengthy branches. Allowing the fruits on the tree to mature. Fruit that has softened implies that it is ripe. The untrained may have trouble determining whether thick-skinned kinds are fully ripe. The fruit must not be picked too soon because if it is, it will shrivel and stop ripening. A few days after they ripen, several cultivars of fruit can be stored in the refrigerator without suffering any harm. Although avocados can be grown on a variety of soil types, they need good drainage because they cannot handle being wet. A 50-inch annual rainfall that is evenly dispersed throughout the year is sufficient. Because avocado wood is brittle and can break, areas with strong winds should be avoided. Because avocado trees are susceptible to sodium chloride (salt), areas along the coast are also unattractive<sup>16</sup>.



**Fig. 2: *Persea Americana***

- **Taxonomy Classification<sup>17</sup>**

Kingdom : Plantae  
 Subkingdom : Tracheobionta  
 Division : Magnoliophyta  
 Class : Magnoliopsida  
 Subclass : Magnoliidae  
 Order : Laurales  
 Family : Lauraceae  
 Genus : *Persea* Mill  
 Species : *Persea americana* Mil  
 Common name : Avocado Pear

- **Vernacular names<sup>18</sup>**

Avocado (En),  
 Avocatier (Fr),  
 Indonesia: adpukat, avokad,  
 Malaysia: avokado, apukado,  
 Philippines: avocado,  
 Thailand: awokado

- **Description:**

The shape of the leaves is round oval and ovate and about length in 3-10 inches. The avocado flower is small and greenish in color. Flowers have both male and female parts. The shape of avocado fruit may be round, ovate or pear-shaped and the skin of the fruit is different in color and appearance in all varieties<sup>16</sup>. The skin may seem yellow-green, purplish-red, or black, be more flexible, or be rough. When ripped, the avocado fruit's flesh has an oily texture and a yellow-green to bright-yellowish hue. However, the inside surface is fibrous<sup>19</sup>. Each avocado fruit has a single big seed, which is ovate or oval in shape and contributes 10 to 25% of the fruit's weight.

### Phytochemical Screening

The active phytochemical elements of any plant species determine its antibacterial action. As a result, phytochemical screening serves as a reference for determining antibacterial activity. Alkaloids, tannins, flavonoids, and phenolic compounds are the most potent phytochemicals found in the plant (Hill, 1952; Ali et al., 2001). These phytochemicals are commonly referred to as secondary plant metabolites<sup>20</sup>. Non-nutritive secondary metabolites with defensive or disease-preventive qualities are known as phytochemicals. Plants produce these compounds in order to defend themselves. Phytochemicals are not essential for immediate plant survival, but are synthesized by plants to boost their own fitness for optimal survival. They also safeguard the plants against environmental threats such as pollution, stress, draughts, and UV radiation.

Every plant in the nature has a few phytochemicals or more. Some of them may be poisons, but the majority have been found to be beneficial in one or more disorders. Phytochemicals are found in many parts of plants, including leaves, fruits, seeds, flowers, stems, and roots, however stem barks and root barks contain the most phytochemicals. They may be essential components of several Ayurvedic rasayanas and Unani medications because of this<sup>21</sup>.

## Anti-Microbial Test

The term "antimicrobial activity" refers to all active principles (agents) that are capable of preventing the development of microbial colonies, inhibiting the growth of microorganisms, and even destroying them<sup>22</sup>.

The process of killing or suppressing disease-causing bacteria is referred to as antimicrobial activity. Antimicrobial agents of various types are utilized for this purpose. Antimicrobials can be antibacterial, antifungal, or antiviral in nature. They all have various ways of action for suppressing the virus<sup>23</sup>. While some bioassays, like disk-diffusion, well diffusion, and broth or agar dilution, are well known and frequently used, others, like flow cytometric and bioluminescent methods, are less popular because they need specialized equipment and additional testing for reproducibility and standardization even though they can quickly show the effects of the antimicrobial agent and give insight into how they affect cell viability and damage<sup>24</sup>.

## MATERIALS AND METHODS

### 1. Collection and authentication of the selected medicinal plant specimen:

The Fruit *Persea americana* collected from the fruit market of Gondia district, Maharashtra, India. Herbarium sheet of plant specimen is certified by Department of Botany, DB Science, Gondia.

### 2. Preparation of Plant Extract

#### Reetha (*Sapindus mukorossi*) Extract<sup>25</sup>

Combine 15-20 soap nuts (seeds removed) and 6 cups water in a large vessel/pan. Let it boil. Once it starts boiling, decrease the flame and reduced it upto 4 cups. The water should become dark after 30 minutes of simmering. Take it off the heat and cover it. Gently squeeze the soap nuts with your fingers when the liquid has totally cooled to release more soap. Leave it overnight, Next Day Strain the liquid & pour it into a container.

#### Avocado (*Persea Americana*) Extract

The collected fruits are cut into little pieces, totally dried, and grind into a coarse powder using an appropriate homogenizer. After homogenization, weighed 30g crude powder and allowed for successive extraction with a 9:1 ratio of organic solvents such as methanol and water by using the Soxhlet Apparatus. The mixture was heated on heating mantle at 60°C for 8-10 successive cycles. The extract was collected and evaporate on a water bath at atmospheric pressure. Lastly, the extracted materials are subjected to phytochemical analysis and antimicrobial testing.

### 3. Preliminary phytochemical screening of the extracts<sup>26, 27</sup>

By using established techniques, a preliminary analysis of extracts was conducted to determine the presence of several phytoconstituents. The findings of the subsequent chemical tests were compiled in Table 2.

### 4. Formulation of Avocado Herbal Hand Wash<sup>28</sup>

- Herbal Hand Wash Gel was made by soaking Carbopol940 in 15ml distilled water overnight as a gelling agent.
- Avocado Extract, Lemon juice and Reetha extract along with castor and mentha oil were measured accurately and dissolved by gentle heating.
- After heating, keep the solution aside for sometimes.
- The required quantity of Glycerin were mixed in above aqueous phase with continuous stirring.
- A mechanical stirrer was used to stir the swollen polymer (940) in order to ensure that the polymer was distributed uniformly.
- Then add into the mixture until form homogeneous gel, then lastly add required amount of lavender oil as a fragrance.
- Stored in well closed container and labeled it.

**Table 1: Formulation of Avocado Herbal Hand Wash**

S.no	Ingredients	Quantity	Uses
1	Avocado extract	20 ml	Anti-microbial agent
2	Reetha extract	40 ml	Foaming Agent
3	Lemon juice	5 ml	Antiseptic
4	glycerin	5ml	Moisturizing Agent
5	Castor oil	0.5 ml	Preservatives
6	Mentha oil	1 ml	Cooling Agent
7	Carbopol 940	1 gm	Thickening agent
8	Lavender oil	2 ml	Fragrance
9	Deionized water	Upto 100 ml	vehicle

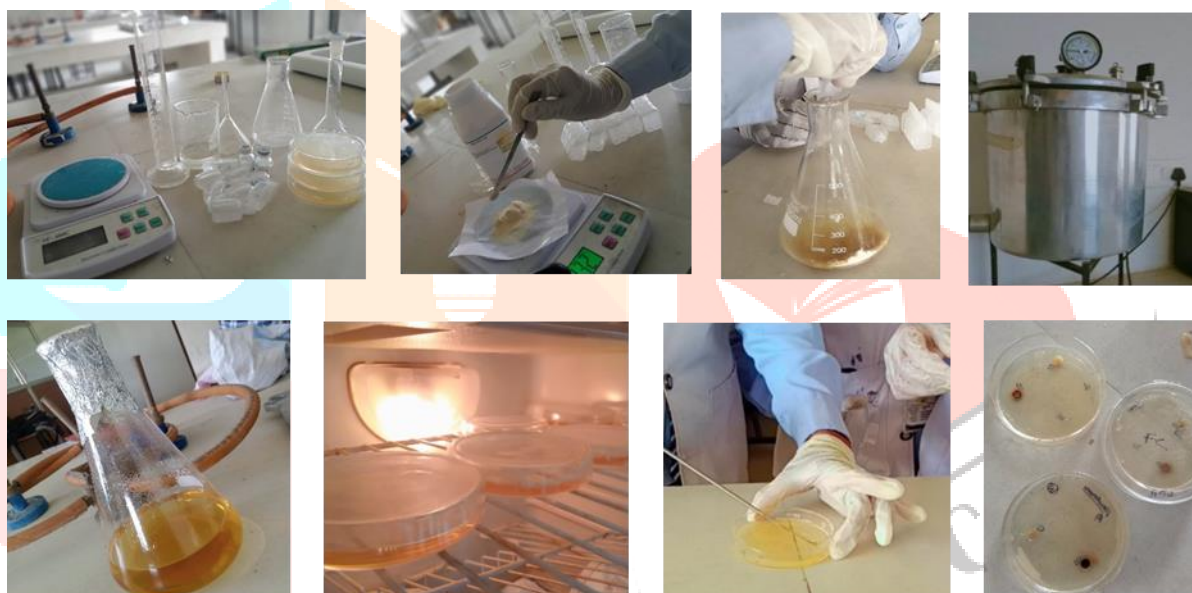
### 5. Evaluation of herbal avocado fruit hand wash<sup>29</sup>

Prepared formulation of Avocado Herbal Hand wash was subjected to following evaluation parameters:

- a) **Organoleptic Evaluation:** Parameters like color, odour, texture was carried out, Color and texture were evaluated by visual and touch sensation respectively. The formulation was sensed in order to examine the odour.
- b) **Appearance and Homogeneity:** It was evaluated by visual inspection.
- c) **Grittiness:** 1ml of was taken on finger tips and rubbed between two fingertips, then the formulation was evaluated.
- d) **Skin Irritation Test:** The Skin Irritation Test was performed by applying Avocado herbal Hand wash to the skin and leaving it for 30 minutes. After 30 minutes, examine the skin for any itching, rashes, or redness using sensory and visual inspection.
- e) **pH:** 1gm of Sample of Avocado herbal Hand wash Gel was taken and dissolved it into 100ml distilled water. The pH solution was measured by standardized digital pH meter.
- f) **Spread ability:** 0.5gm of Avocado herbal Hand wash Gel was put between two slides and left for around 5 minutes with no further spreading expected. The diameter of the spreaded circle was measured in centimeters and used to compare spread ability.
- g) **Viscosity:** The viscosity of Avocado herbal Hand wash Gel was determined by using Ostwald viscometer.
- h) **Foam Height:** One gramme of Avocado herbal Hand wash Gel was spread in 50ml of purified water. The dispersion was poured into a measuring cylinder. With water, the volume was increased to 100ml. This solution is placed in 10 test tubes in a sequence of successive portions of 1, 2, 3... 10ml, and the remaining capacity is filled with water to a volume of 10ml. The test tubes were then shaken for 15 seconds. The test tube is then left to stand for 5 minutes. The height of the foam was also measured.
- i) **Foam Retention:** 25ml Avocado herbal Hand wash Gel was poured into a 100ml measuring cylinder and shaken 10 times. The volume of foam was monitored at one-minute intervals for four minutes. For at least 5 minutes, foam retention should be stable.
- j) **Stability:** Avocado herbal Hand wash Gel formulation stability studies were conducted by storing it at different temperatures such as 40°C, 25°C, and 37°C for one week. During the stability tests, no color change or phase separation was noticed in the prepared hand wash.
- k) **Dirt dispersion test:** 1ml of Avocado herbal Hand wash Gel was added in a test tube containing 10ml of distilled water. A drop of Indian ink was added; the test tube was stoppered and shaken. The ink content of the foam was evaluated to be none, light, moderate, or substantial.

**Screening Antibacterial activity of extracts will be performed by Agar Well Diffusion method.**

- Dissolve 38 g of dehydrated Mueller Hinton Agar (MHA) medium into 1 liter distilled water. Shake and heat to dissolve completely.
- Now autoclave the medium at 121 °C temperature and 15 psi for 15 min.
- Transfer the medium in a sterile petri dish, then allow it to cool and solidify.
- A fresh MHA plate with sample name, bacteria name etc.
- Bring the sterile inoculating loop and insert into the bacterial culture [*Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Staphylococcus aureus*] hold for 5 sec.
- Now take out the inoculating loop from culture tube and inoculate MHA plate in zigzag style.
- Inoculation should be done by the rotating the MHA plate occasionally.
- Subsequently, wells of 8 mm diameter were punched into the agar medium by metallic borer and well filled with plant extract and antibiotic (streptomycin).
- The petri dishes incubated at 37oC for 24 hours. After 24 hr., the zone of inhibition was measured and compared with the zone of inhibition of control (streptomycin).

**Fig. 3 Preparation of Agar Plate**

**RESULTS AND DISCUSSION**

All the observation data for evaluation of Hand wash presented as following Table

**Table 2 Phytochemical Screening**

Plant constituent's	Test/Reagent	Result
Sterols	Salkowski	+
	Liebermann's	-
	Liebermann-Burchard	-
Alkaloids	Dragendorff's	+
	Hager's	+
	Mayer's	-
	Wagner's	+
Saponins	Foam test	+
Tannins	Ferric Chloride	-
	Lead acetate	+
	Pot. Dichromate	+
Flavonoids	Shinoda test	+
Carbohydrates	Molisch	+
	Fehling's	-
	Barfoed's	-
Amino acid	Ninhydrine	-
Protein	Million's	-
	Biuret	-
	Xanthoproteic	+

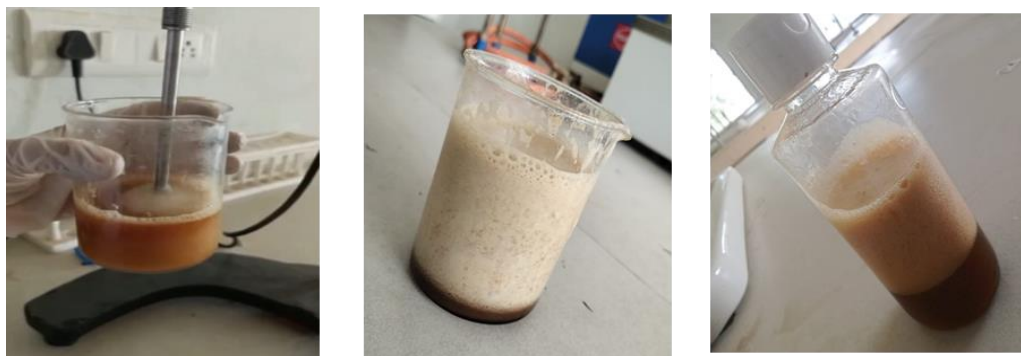
Above Table 2 of Preliminary phytochemical screening of the Avocado extract shows the presences of Sterol, Alkaloid, Saponins, Tannins, Flavonoid, Carbohydrate and protein.

**Evaluation Parameters:** The prepared formulations Herbal Avocado hand wash were subjected to physical evaluation and other evaluation parameters.

**Table 3 Evaluation Parameter**

Sr no.	Evaluation Parameter	Result
1	Color	Brownish
2	Odour	Lavender
3	Texture	Smooth
4	Appearance	Opaque
5	Homogeneity	Homogeneous
6	Grittiness	Non gritty
7	Skin Irritation Test	No irritation
8	PH	5.6
9	Spreadability	4 cm
10	Viscosity	40 cps
11	Foam Height	2.2 cm
12	Foam Retention	12.2 ml
13	Stability	Stable
14	Dirt dispersion test	Light



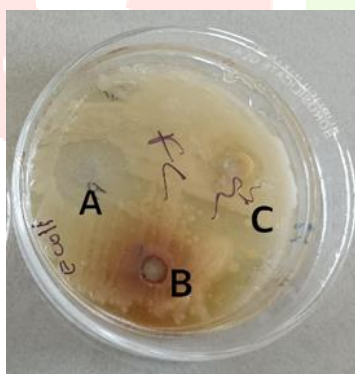


**FIG. 4: Formulated Avocado Herbal Handwash**

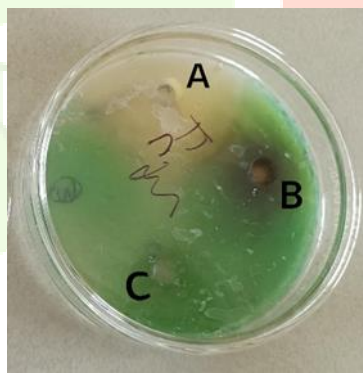
**Antimicrobial activity:** By using the Agar well diffusion method, the anti-microbial effectiveness of the developed Avocado Herbal Hand Wash was evaluated against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The results of this method showed that the herbal hand wash prepared from methanol extract of the combined plant materials has shown significant antimicrobial activity. The data of zone of inhibition of formulations is shown in below Table 4.

**Table 4: Zone of Inhibition of Sample**

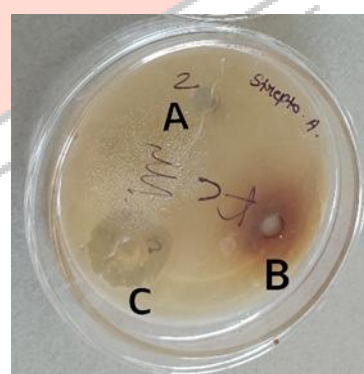
Sr no	Sample	<i>E. Coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>
A	Antibiotic	2.5 cm	2.9 cm	No Zone
B	Pure Extract	1.6 cm	1.5 cm	2.3 cm
C	Formulated Hand wash	1.9 cm	1 cm	2.5 cm



**Fig. 5: *E. Coli***



**Fig. 6: *P. Aeruginosa***



**Fig. 7: *S Aureus***

## CONCLUSION

The active constituents (saponin, flavonoids, tannin, carbohydrates, and proteins) present in the methanolic extract of Avocado Fruit Extract showed superior inhibition against various Gramme positive and Gramme negative bacteria than synthetic antibacterial agents present in commercially available antiseptic liquid soaps in the current study. As a result, these chemicals might be isolated and combined into soap bases to create superior antibacterial soap with significant activity and minimal to no adverse effects. The WHO estimates that 80% of Asian countries already use herbal medicine for primary aspects of primary health care, which includes the creation of hand wash. Hence, it can be concluded that Avocado herbal hand wash are far superior to basic soaps or currently available synthetic hand wash due to their constituents and effectiveness on our hands' skin, as well as being acceptable for all skin types. Formulation can also be used on a regular basis to improve the cleanliness of healthy children and adults. Future challenges include more pharmacological evaluations, toxicological research, and the probable isolation of a therapeutic antibiotic from this plant.

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