Formulation and Evaluation of Herbal Hand Wash Gel for Antimicrobial Activity using *Lagenaria siceraria* and *Aloe barbadensis miller* leaves

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ABSTRACT:

The global significance of herbal products has escalated, driven by their medicinal and economic value. However, concerns regarding their quality, safety, and efficacy persist in both industrialized and developing nations. This study aimed to formulate and evaluate herbal hand wash formulation utilizing *Lagenaria siceraria* leaves and *Aloe barbadensis miller* leaves gel, a plant from the Cucurbitaceae and Liliaceae family, which has been traditionally employed in various medicinal systems to treat human ailments. The ethanolic extract of *L. siceraria* leaves and pure gel of *Aloe barbadensis miller* was assessed for antimicrobial activity against a broad spectrum of gram-positive and gram-negative bacteria using the cup-plate and disc diffusion methods. Physicochemical parameters, including physical evaluation, pH, foaming retention, foam height, viscosity, and stability, were determined. The formulation's efficacy was compared to the standard antibiotic streptomycin, and the results demonstrated significant antimicrobial activity against Escherichia coli and Staphylococcus aureus.

Key words*:* *Lagenaria siceraria*, *Aloe barabdensis miller*, Herbal hand wash, Antimicrobial

INTRODUCTION:

Herbal medicine, also known as phytotherapy, is the science of using herbal drugs or herbal remedies to heal the ailments(1). Herbal medicine is an incredibly old practice possibly even older than mankind itself(2).

World Health Organization (WHO) specifies that phytomedicines are deemed finished and labeled pharmaceutical products that contain active substances, which may consist of aerial or subterranean parts of plants, and other plant materials. WHO also said that phytomedicine is employed to prevent, diagnose, improvement, cure physical and mental health issues. Traditionally, tinctures, tea, poultices, powders, and other medicinal products were the basis for phytomedicines derived from crude drug. In developing nations across the globe, between 70 % and 80% of primary health care treatments are still provided by herbal remedies. A significant number of primary healthcare services utilize them because herbal drugs have been proven to have no or less adverse effect in addition to being inexpensive and readily accessible(3).

Hand wash:

Hands are the main medium through which all bacteria and infections are transmitted. The most effective, least complicated and affordable way to avoid nosocomial infections is via good hand cleanliness. When a culinary worker taint their hands and subsequently comes into touch with food or drink, bacteria are transmitted from one person to another, leading to the spread of infections. Hand washing is an essential precautionary measure to safeguard the skin against deleterious agents and prevent to extend of numerous contagions. Hand washing eliminates dirt that is visible on hands and lowers the number of dangerous bacteria that might infect food such as Salmonella typhi and E. coli(4).

Hand hygiene:

Hygiene is defined as maintenance criteria for cleanliness practices which are important in maintenance of health(5). Hand hygiene refers to the standardized protocol of hand cleansing with water, soap, or designated liquid cleansing agents(6). The benefit of hand washing is that it cleanses the hands of pathogens (bacteria and viruses), harmful chemicals and dirt. Individuals employed in healthcare facilities, restaurants are involved in the preparation and service of food for the public should prioritize meticulous hand hygiene practices(7). The inaugural Global Patient Safety Challenge initiated by the World Health Organization, commonly known as “Clean Care is Safer Care” based on science, user-centered concept, “My five moments for hand hygiene,” has been created for teaching, measuring and reporting hand hygiene compliance(8). The first national hand hygiene recommendations were released in the 1980s, and several more were released in several nations in more recent years(9).

Steps of hand washing:

According to WHO six steps techniques for effective hand washing are as follows:

1. Step-1: Wet hands and apply herbal hand wash and rub palms until bubbles develop.
2. Step-2: Gently rubs the back of the opposite hand with each palm.
3. Step-3: Rub each hand's fingers together.
4. Step-4: 2. Interlock your fingers and rub your hands together in a circular motion**.**
5. Step-5: Give each thumb a gentle rub.
6. Step-6: Give your palms a circular massage. Next, wash and dry your hands(6).

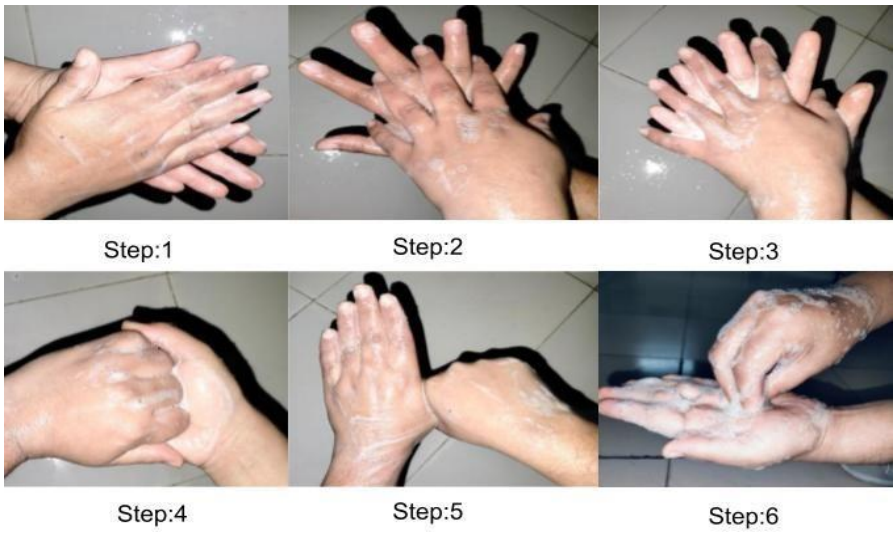


Fig-1: Steps of hand washing

Lagenaria Siceraria:

Lagenaria siceraria (Molina) standl is also referred to as bottle gourd. *Lagenaria siceraria* is a member of the Cucurbitaceae family. The Cucurbitaceae family, comprising various species of pumpkins, melons, and gourds, is collectively known by these names. There are 825 species and 118 genera in this family, most of which are found in the world's warmer climates. *The Lagenaria* species is the most widely used plant in the Cucurbitaceae family. The nomenclature of the genus Lagenaria, which includes the bottle gourd, is derived from the Latin term "Lagena," literally meaning "bottle". Bottle gourd, which is the oldest cultivated plant and are used for their nutritional and therapeutic features, make up the majority of economically significant domesticated species. Bottle gourds belong to the genus Lagenaria, which is distinguished by two main characteristics: single, chalky white blooms and soft, many-seeded fruits. The female and male flowers open simultaneously. Male flowers have a short lifespan because they only bloom for a few hours before the petals die. *L. siceraria are* available in two varieties: sweet and bitter. Botanically, they are both members of the same genus; the latter is known as Iksuaku, Katutumbi and Mahapala. The bitter

species is preferred for medicinal purposes, the sweet type is typically utilised as a vegetable (10). Bottle gourds can be used for medicine, food and a wide range of musical instruments(11).

Fruits are large, varying, cylindrical, flask-shaped, green, and densely hairy and mature to a pale brown or yellowish color. Stem upto 5 cm long, climbing, ribbed, angular, brittle, and thick and softly hair. Leaves are simple and nonaromatic, size upto 400 mm in length and width. They possess a long petiole, are 5- lobed, pubscent and covered with soft hair and ovate, kidney shaped or heart shaped in outline, undivided, angular 3 to 7 lobed. The leaves stalks can reach upto 300 mm in legth, thick, hollow, densely hair with two small lateral glands at the leaves base. Flowers are cream and with the darker veins and have a pale-yellow base. Each flower has five crisped, obovate petals that can grow up to 45 mm long. They open in the evening and wilt shortly thereafter. Seeds are I and irregular and wrinkled, embedded in a spongy pulp & compressed, featuring two flat facial ridges(12).

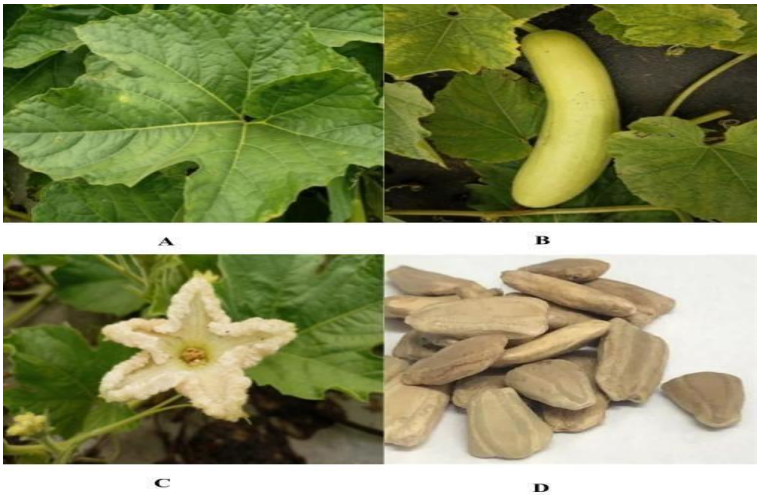


Fig-2: Different parts of *Lagenaria siceraria* (A) Leaves (B) Fruit (C) Flower (D) Seed

Aloe Barbadensis Miller:

The nomenclature of Aloe vera originates from the Arabic term "Alloeh," signifying "bitter," and the Latin word "vera," denoting "true"(13). *Aloe barbadensis Miller* is the most physiologically active plant among the 400 species with its origin in the African region. The genus Aloe is classified under the family Liliaceae. Plant height about 80 to 100 cm; matures in 4 to 6 years; under ideal circumstances, persists for almost 50 years(14). Typical xerophyte aloe vera has thick, meaty, spiky leaves with unique cuticularization(15). Aloe vera's thick leaves store water. The plant has a pH of 4.5 and consists of 99.3% water and 0.7% solid matter, primarily glucose and mannose. The gel contains a synergistic combination of sugars, enzymes, and amino acids, contribute to its unique effectiveness as a skin care product(16). . The gel is a colorless, viscous, watery-thin liquid that contains anthraquinone, glycoprotein, prostaglandins, gamma-lanoline acid and muco polysaccharides which are primarily

responsive for its antibacterial, antiviral and antifungal activities. Because of its ability to hold water, the gel not only makes the skin more hydrated but also promotes cell growth, which helps the skin heal from damage(15). Egyptians referred to aloe as "the plant of immortality." It is frequently incorporated into dermatological treatment regimens(13).

Fig-3: Leaves of *Aloe Barbadensis Miller*

METHOD AND MATERIAL:

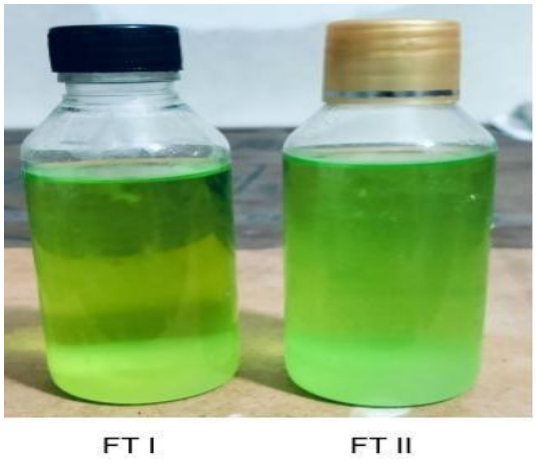
Plant material: *Lagenaria siceraria* (Monila) standley and *Aloe barbadensis miller* leaves has been obtained from the local area of Lucknow and Paratapgarh (Uttar Pradesh).

Preparation of extract: The collected plant leaves of *Lagenaria siceraria* were air dried. Dried leaves put into the grinder jar for size reduction and afterwards sieve no.40 used to get accurate coarse powder. As a result, the extraction procedure is made easier. Plant powder is placed in the maceration apparatus and extracted continuously with ethanol at room temperature for 3 day. After 3 days, the extract underwent filtration using filter paper. Residue further extracted using the same procedure until all components have been isolated and filtrate evaporated to dryness. Extract stored at 20 °C(17).
Gel of *Aloe barbadensis miller* used as such(16).

Fig-4: Extraction of *L. siceraria* leaves

Formulation of herbal hand wash gel:

- An herbal hand wash gel was created by soaking carbopol-934 a gelling agent in distil water for the entire night and slowly stirring the mixture to create a homogenousdispersion.
- Distilled water was used to dissolve the required amount of SLS, and then glycerin was added to the solution while stirring continuously.
- Distilled water was used to dissolve methyl paraben.
- The *Lagenaria siceraria* leaf extract and *Aloe barbadensis Miller* gel were blended into the mixture to achieve a consistent gel texture, after which triethanolamine .
- Upon completion, the formulation was transferred to a well-sealed container for storage and subsequent analytical testing(18).



FT I =Formulation 1 and FT II= Formulation 2

Fig-5: Herbal hand wash gel

Table 1: Compositions of herbal hand wash gel

S.No.	Ingredients	FT 1	FT 2
1.	*Lagenaria siceraria* leaves extarct	40 mg	50 mg
2.	Aloe vera gel	10 ml	10 ml
3.	Carbopol- 934	0.5 gm	0.5 gm
4.	SLS	2 gm	2 gm
5.	Glycerin	2.5 ml	2.5 ml
6.	Methyl paraben	0.5 gm	0.5 gm
7.	Triethanolamine	q.s.	q.s.
8.	Distilled water	Up to 100 ml	Up to 100 ml

EVALUATION PARAMETER:

1. **Physical evaluation:**
   A visual examination of the test formulation was carried out. The evaluation parameters were color, texture, appearance and homogeneity**(19)**.
2. **pH:**
   Take 100 ml distilled water and 1 ml test formulation was mixed. The pH was evaluated with the help of pH paper**(20)**.
3. **Viscosity:**
   Viscosity evaluation of test formulation was used digital Brookfield viscometer(19).
4. **Foam retention:**
   A mixture of 250 ml water and 50 ml test formulation was prepared in a measuring cylinder and agitated ten times. Foam production was monitored and recorded at 1-minute intervals for 4 minutes(19).
5. **Foam height:**
   For foam evaluation, 1ml of test formulation was mixed with 50ml of distilled water in a cylinder, shaken for 15 seconds, and the foam height subsequently measured(21).
6. **Skin irritation:**
   Apply hand wash gel on the skin, leaving it to rest for 30 minutes. After washing, use your senses to look for any redness, rashes or itching on the surface of your skin(18).
7. **Stability:**
   Stability tests were performed by being stored at various temperatures like 25°C, 37°C, and 40°C for one week. In this investigation, the hand wash's formulation did not exhibit any color change or phase separation(18).

ANTIMICROBIAL SCREENING:

1. In Vitro Metho

Cup plate method:

Preparation of stock and standard solutions:

Test and standard drug were dissolved in dimethyl sulphoxide (DMSO) in volumetric flasks to yield a solution at a concentration of 1mg/ml.

Procedure:

Nutrient agar medium was used for antimicrobial assay. The nutrient agar was produced in accordance with the requirements and autoclaved for 45 minutes at 121°C. After that at 37°C nutrient media were cooled.

Table 2: Composition of nutrients agar media

S. No.	Components	Quantity
1.	Beef extract	0.3 gm
2.	Peptone	0.5 gm
3.	Sodium chloride	0.5 gm
4.	Agar	2.0 gm
5.	Distilled water	100 ml

Fig-6: Composition of nutrient agar media

The standard and test drug were formulated using dimethyl sulphoxide. Streptomycin served as the standard reference drug. A sterile Pasteur pipette was used to inoculate the petri plates holding 25 millimeters of sterile nutritional agar with standardized inoculum. A steel borer

was used to create 8 mm diameter wells in the center of each plate. 0.2ml of different test and standard drug were aseptically poured into each of these wells. The test substances were exposed to the medium at ambient conditions (room temperature) for 1 hour, enabling diffusion. Incubation of the plates was conducted at 37°C ± 1°C for a period of 18 hours to support bacterial development. The zone of inhibition around the wells was utilized as an indicator to evaluate the antimicrobial activity of the test and standard drugs. To ensure reliability, the test was repeated three times, and the average value was evaluated(14).

Disc diffusion method:

For the disc diffusion assay, the agar medium was reconstituted with water and subsequently sterilized. The mixture was then inoculated onto pre-labelled Petri plates, specifically assigned as Standard drug, Marketed Formulation, Test Formulation I, Test Formulation II. The experimental studies employed bacterial strains of *Escherichia coli* and *Staphylococcus aureus*. The inoculated Petri plates were subsequently positioned in an incubator to facilitate bacterial growth. Discs with a diameter of 5mm were exercising and sterilized, soaked in 0.1µl prepared hand wash and marketed formulation, and then deposited onto the Petri plates. Upon completion of the 24-hour incubation, the Petri plates were removed and analyzed for inhibition zones(22).

2. In Vivo Method

Experimental animal:

Female Swiss albino mice, weighing between 20-30 grams, were procured from the Animal House Facility at the Institute of Pharmaceutical Sciences and Research, Sohramau, Unnao, Uttar Pradesh.
All experiment animals were housed in polypropylene cages with three female mice in one cage at a 22±3ºC temperature and humidity 40-65% with a 12-hour light and dark cycle.

Acute dermal toxicity:

According to the OECD guideline, the test on Swiss albino mice was performed. The animals were acclimated to the conditions in the animal house for 14 days at a 25°C temperature with 12 hours of light and 12 hours dark. Standard laboratory hygienic condition & operation were confirmed. Animals were fed with hybrid feed and deionized water. The animal was divided into three groups with 3 animals in each for test formulation 1, test formulation 2 and control group. The dorsal surface of the mice's trunks was then denuded of fur at least 10% of body surface area by trimming and stripping about 24 hours before the test formulation applied and taking care must avoid abrading the skin. After shaving, deionized water was used to clean the skin’s surface**(23)**.

Application of test formulation:

According to OECD guideline 402, the test hand wash formulation FT1 and FT2 and negative control 0.5 ml were applied thin and uniform film over 4 cm square shaved skin surface of mice in each group for 14 days and covered using nonirritating adhesive tape. The following variables were observed like skin irritation and defeating of the skin, any adverse effects like toxicity on mucus membrane, eye, body weight and respiration etc.(24). The skin patches were removed and macroscopically examined. For macroscopically examination, sample skin patches and fragments are fixed in 10% of neutral formalin solution (pH-7.4) & implant in paraffin for histological inspection(23).

Fig-7: Application of test formulation 1 & 2 and control group

Histopathological Analysis:

Swiss albino mice (female) were anaesthetized on the 14th day and skin and skin patches were removed and macroscopically analyzed. Skin patches and pieces were preserved into 10% formalin solution (ph- 7.4) and encased in paraffin. Haematoxylin –eosin was used to color a 4µm section before being collected for microscopic analysis(23).

RESULT:

Evaluation Parameter:

1. **Physical appearance:**

The FT1 and FT 2 herbal hand wash gel were observed to possess a light green color. Furthermore, upon tropical application to the skin, both formulations elicited a smooth and cooling sensation.

2. **pH:**

The pH of both formulations was between the ranges of the skin ph.

Table 3: pH of herbal hand washes gel

S.no.	Formulation	pH of formulation
1.	Formulation 1	4.8
2.	Formulation 2	4.9

3. **Viscosity:**

The viscosity of formulation 1 and formulation 2 was identified using digital Brookfield viscometer are given in Table 4.

Table 4: Viscosity of formulation 1 and formulation 2

S.No.	Formulation	Viscosity
1.	Formulation 1	52 cps
2.	Formulation 2	cps

4. **Foam height:**

Foam height of both formulations is given in Table 5.

Table 5: Foam height of formulation 1 and 2

S.no.	FT 1	FT 2
1.	15.6cm	18cm

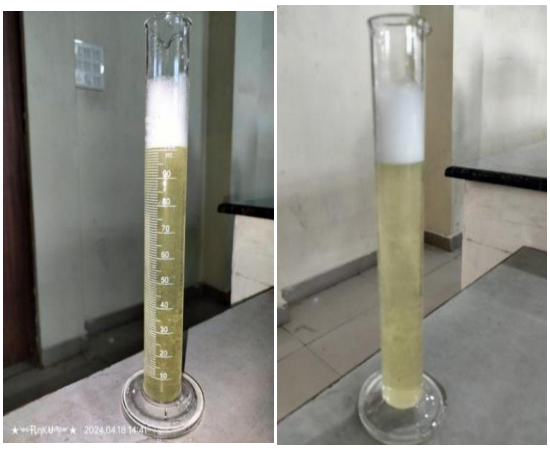
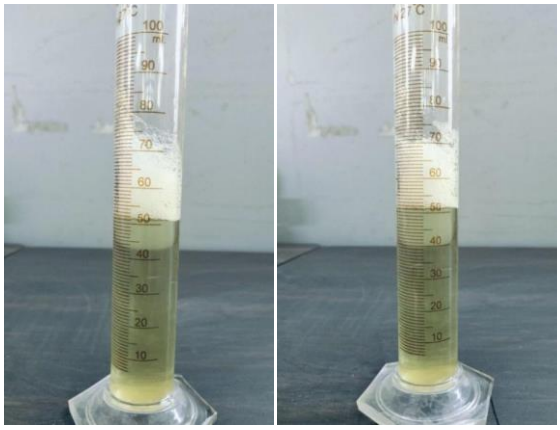


Fig-9: Foam height of FT1 and FT2

5. **Foam retention:**

The volume of foam of herbal hand wash gel FT 1 and FT 2 at one-minute intervals were found to be 25 ml and 22 ml, consequently.



FT 1 **FT 2**

Fig-10: Foam retention of herbal hand wash gel

6. **Skin irritation:**

No sensation of irritation was experienced on the skin, and there were no visible signs of redness.

7. **Stability:**

There was no change visible in color and phase diversion in both formulations.

ANTIMICROBIAL ACTIVITY:

1. Cup plate method:

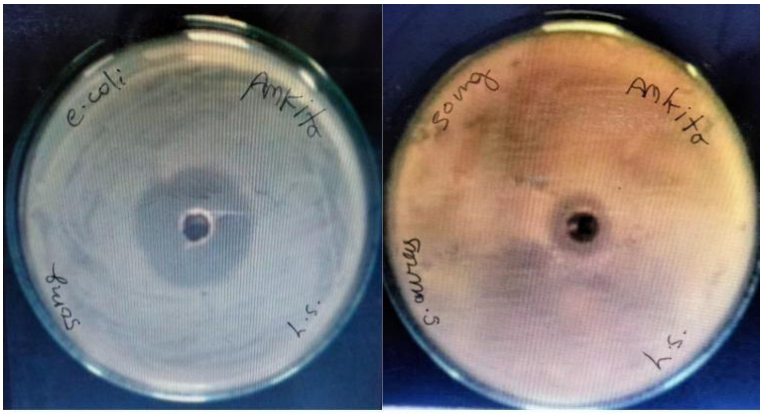
The antimicrobial efficacy as measured by the zone of inhibition of *Lagenaria siceraria* molina standl leaves extract and *Aloe barbadensis miller* leaves gel was tested against *Staphylococcus aureus* and *E.coli.*

Ethanolic extract of plant and gel indicated the antimicrobial efficacy against the bacteria. The ethanolic extract of *L.S.* leaves and *Aloe barbadensis miller* gel showed more activity on *E.coli* than *S.aureus.*

Streptomycin was used as a standard drug for antimicrobial activity.

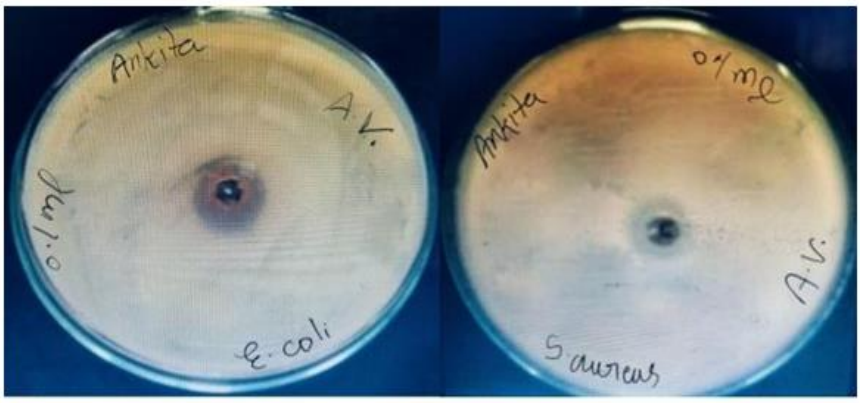
Table 6: Zone of inhibition of *Lagenaria siceraria* leaves, *Aloe barbadensismiller* and Streptomycin

S.No.	Sample	Conc.mg/ml	Zone of inhibition (mm)	
			Mean±SEM)	S.aureus(Mean ±SEM)
1.	*Lagenaria siceraria* leaves extract	50mg	24.4±0.5***	16.5±0.5**
2.	*Aloe barbadensis miller* leaves gel	0.1ml	12.6±0.7**	10.3±0.7*
3.	Streptomycin	50 mg	32.5±0.5	30.1±0.7
4.	Control	-	-	-



A B

Fig-11: Zone of inhibition of L.S. leaves extract on *E.coli* and S. aureus bacteria



A B

Fig-12: Zone of inhibition of *Aloe barbadensis miller* leaves gel on *E.coli* and S. *aureus* bacteria

Fig-13: Zone of inhibition of Streptomycin on *E.coli* and *S. aureus* bacteria

A B

Fig-14: Zone of inhibition of control group on *E.coli* and *S.aureus* bacteria

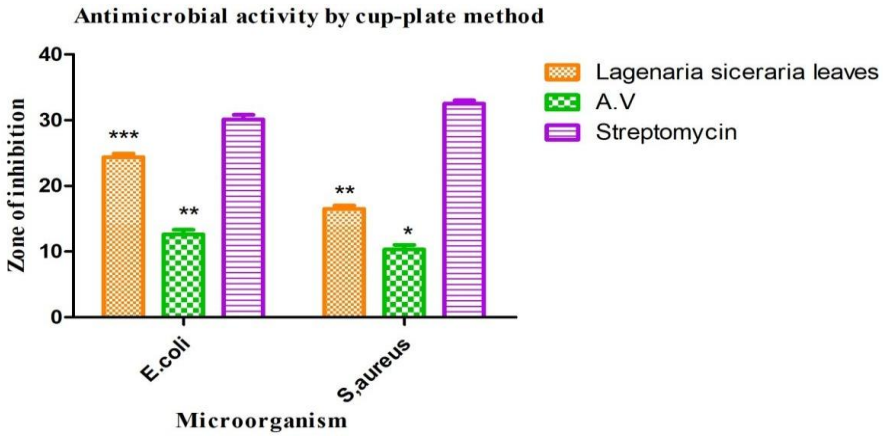


Fig-15: Zone of inhibition graph of *L. siceraria* leaves extract, *Aloe barbadensis miller* leaves gel and Streptomycin by one-way Anova

Table 7: Zone of inhibition of herbal hand wash gel and marketed formulation

S.No.	Sample	Zone of inhibition in mm	
		E. coli (Mean±SEM)	S.aureus (Mean ± SEM)
1.	Formulation 1	9±0.5	8.5±0.5
2.	Formulation 2	15.2±0.5	12.6±0.5
3.	Marketed Formulation	25±0.5	19.1±0.5

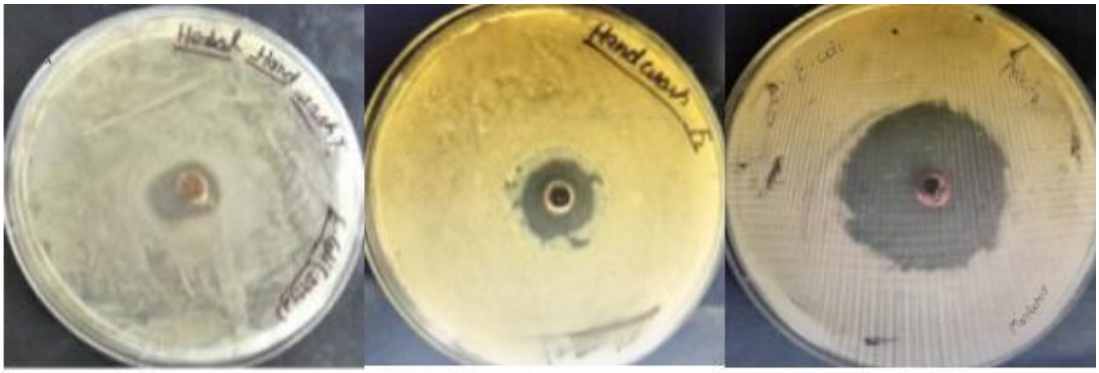


Fig-16: Zone of inhibition of herbal hand wash gel and marketed formulation againt E.coli bacteria

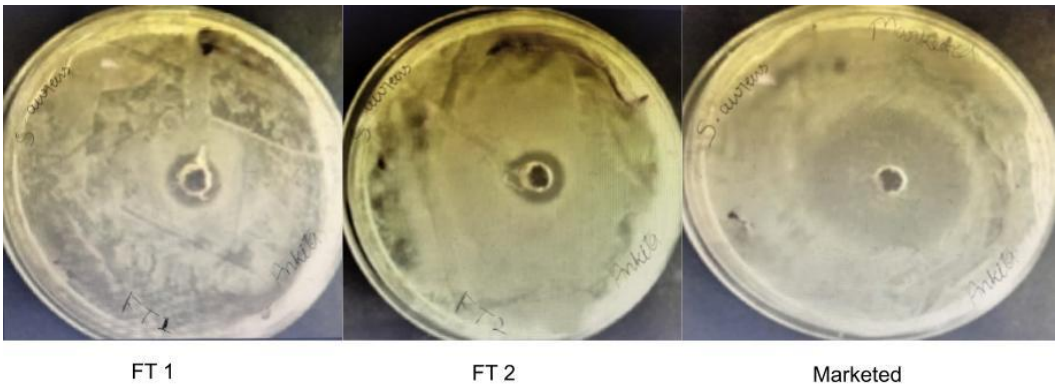


Fig-17: Zone of inhibition of herbal hand wash gel and marketed formulation againt S.aureus bacteria

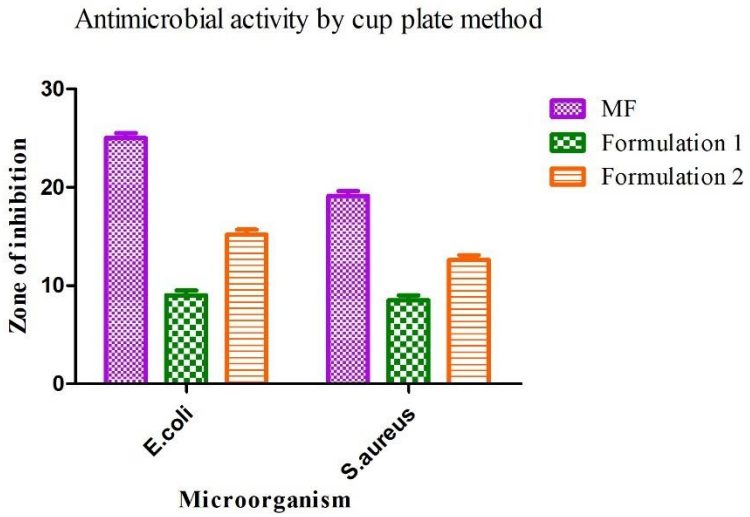


Fig-18: Zone of inhibition graph of Herbal hand wash gel and Marketed formulation by one-way Anova

2. Disc diffusion method:

Herbal hand wash gel Formulation 1 and 2 both indicated the activity for *S.aureus* and *E. coli* bacteria but formulation 2 showed more activity on *E.coli* strain than *S.aureus.* Zone of inhibition data represented in table no.7.14 are given.

Table 8: Zone of inhibition of Formulation 1, Formulation 2, Marketedformulation and Control

S.No.	Sample	Zone of inhibition(mm)	
		E.coli(Mean ± SEM)	S.aureus(Mean ± SEM)
1	Formulation 1	8.50±0.4	7.10±0.5
2	Formulation 2	14±0.5	12±1
3	Marketed formulation	21±0.7	20.8±0.5
4	Control	-	-

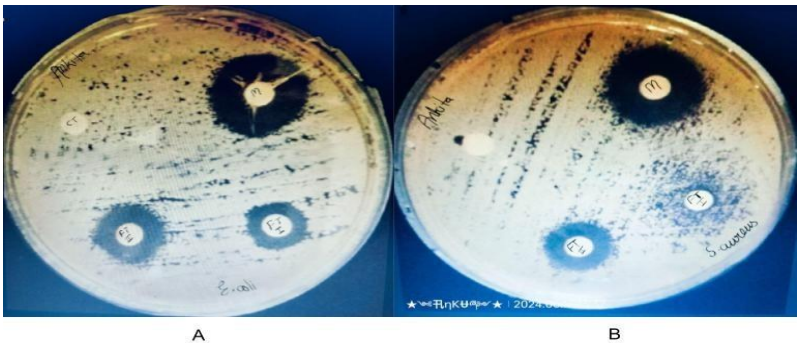


Fig-19: Zone of inhibition of Formulation1, Formulation 2, Marketed formulation and Control

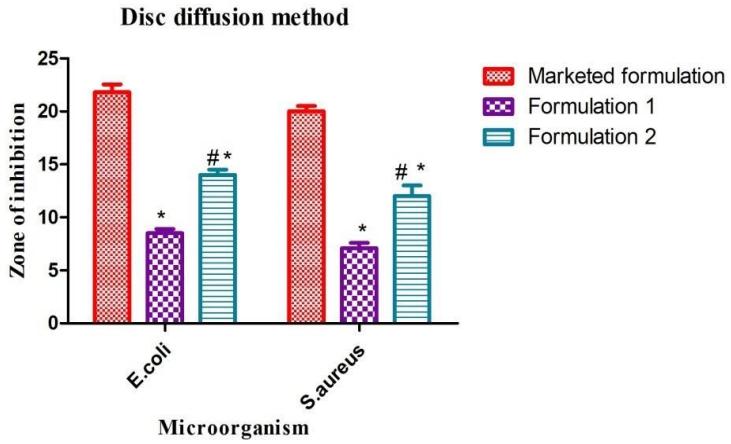


Fig-20: Zone of inhibition graph of marketed formulation andTest formulation 1 & 2 by one-way Anova

CONCLUSION:

In conclusion, our result for herbal hand wash gel using *Lagenaria siceraria* and *Aloe barbadensis miller* leaves extract for antimicrobial activity effectively reduced the microbial growth. Its evaluation parameter range was like standard range. The acute dermal toxicity for herbal hand wash gel dose at 0.5 ml on Swiss albino mice revealed no skin allergic reaction and indicating that product was safe for skin under the topical condition. The result showed that the herbal hand wash gel does not trigger any skin irritation or allergic reaction when applied on the mice skin. Overall, herbal hand wash gel for antimicrobial activity is an important process to promoting health and preventing the spread of diseases.

References:

1. Meuss A. Herbal medicine. Curr Sci. 2000;78:35–9.
2. Tyler VE. Herbal medicine: from the past to the future. Public Health Nutr. 2000 Dec;3(4a):447–52.

3. Agarwal A. Critical issues in quality control of herbal products. Pharma times. 2005;37(6):09–11.
4. Shah MA, Natarajan SB, Gousuddin M. Formulation, evaluation, and antibacterial efficiency of herbal hand wash Gel. Int J Pharm Sci. 2014;25(2):120–4.
5. Hagir DN, Otari KV, Surushe PP. Optimization and Characterization of Herbal Hand Sanitizer by Using Drug Parsley. AJPTR. 2020 Jan 20;8(1):9–17.
6. Bahuguna M, Kashyap S. FORMULATION AND EVALUATION OF HAND WASH. World Journal of Pharmaceutical Research. 5(7).
7. Sinha SK, Poudyal S, Khatiwada S, Ahmed S, Chatterjee A, Mohanty J, et al. Formulation and evaluation of herbal handwash using neem and reetha extract.
8. Sax H, Allegranzi B, Chraïti MN, Boyce J, Larson E, Pittet D. The World Health Organization hand hygiene observation method. American journal of infection control. 2009;37(10):827–34.
9. Pittet D, Allegranzi B, Sax H. HISTORY OF HAND HYGIENE. Bennett & Brachman's Hospital Infections. 2007;31.
10. Upaganlawar A, Balaraman R. BOTTLE GOURD (LAGENARIA SICERARIA) "A VEGETABLE FOOD FOR HUMAN HEALTH"- A COMPREHENSIVE REVIEW. 2009;
11. Kumar SC, Paul B, Kumar BB, Shahid-Ud-Daula AFM. Antimicrobial activity of Lagenaria siceraria crude extracts obtained from its flowers. International Journal of Pharmacognosy. 2014;1(12):780–4.
12. Prajapati RP, Kalariya M, Parmar SK, Sheth NR. Phytochemical and pharmacological review of Lagenaria sicereria. Journal of Ayurveda and Integrative Medicine. 2010;1(4):266.
13. Surjushe A, Vasani R, Saple DG. Aloe vera: a short review. Indian journal of dermatology. 2008;53(4):163.
14. Kumar S, Jakhar DS, Singh R. Evaluating antimicrobial activity of Aloe vera plant extract in human life. Biomed J Sci Tech Res. 2017;1:1854–6.
15. Shahzad K, Ahmad R, Nawaz S, Saeed S, Iqbal Z. Comparative antimicrobial activity of Aloe vera gel on microorganisms of public health significance. Pharmacologyonline. 2009;1:416–23.
16. Olaleye MT, Bello-Michael CO. Comparative antimicrobial activities of Aloe vera gel and leaf. African journal of biotechnology [Internet]. 2005 [cited 2023 Oct 29];4(12). Available from: https://www.ajol.info/index.php/ajb/article/view/71436
17. Abdel-moatamed B, Roby M, Elneairy N, Fakhrany AE. Antimicrobial activity of bottle gourd leaf extract and its effect on the quality of food. Fayoum Journal of Agricultural Research and Development. 2021 Jan 1;35(1):57–69.
18. Mane SL, Chandakavthe BN, Atkare A, Kshirsagar A, Waykule N. FORMULATION AND EVALUATION OF HAND WASH GEL USING BLACK SEED OIL. 2022 [cited 2024 Jan 17]; Available from: https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/aae18a8b52208c30b420ee0a5151915d.pdf
19. Bagade PV, Gidde ND, Nadaf SI, Desai PV, Lokhande MS, Salgar LV. Formulation and Evaluation of Gel Based Herbal Hand Wash Using Extracts of Argemone Mexicana. IJPSM. 2021 Jun 30;6(6):28–33.

20. Arjun GG, Muley VR. FORMULATION AND EVALUATION OF HERBAL SOAP OF NEEM AND TULSI. [cited 2024 Feb 20]; Available from: https://www.irjmets.com/uploadedfiles/paper/issue_1_january_2024/48734/final/fin_irjmets1705746760.pdf
21. Kumar DS, Kumar DA. FORMULATION, DEVELOPMENT AND EVALUATION OF NOVEL POLYHERBAL HANDWASH CONTAINING MEDICINAL PLANT: (MIMOSA PUDICA, AZADIRACHTA INDICA, OCIMUM SANCTUM AND GLYCYRRHIZA GLABRA). World Journal of Pharmaceutical Research.
22. Rajalakshmi M. Preparation and testing for the efficacy of polyherbal hand wash. International Journal of Pharmaceutical Research and Life Sciences. 2019;7(1):6–9.
23. Nyigo V, Mdegela R, Mabiki F, Malebo H. Assessment of Dermal Irritation and Acute Toxicity Potential of Extracts from Synadenium glaucescens on Healthy Rabbits, Wistar Albino Rats and Albino Mice. European Journal of Medicinal Plants. 2015 Jan 10;10.
24. Mielke H, Strickland J, Jacobs MN, Mehta JM. Biometrical evaluation of the performance of the revised OECD Test Guideline 402 for assessing acute dermal toxicity. Regulatory Toxicology and Pharmacology. 2017;89:26–39.