IJBPAS, December, 2026, 15(12): 5774-5790

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DEVELOPMENT, PHYSICOCHEMICAL CHARACTERIZATION, AND BIOLOGICAL EVALUATION OF A MULTI-FUNCTIONAL POLYHERBAL ETHANOL-BASED HAND SANITIZING FORMULATION FOR ENHANCED ANTIMICROBIAL AND SKIN-PROTECTIVE ACTIVITY

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Received 20th May 2026; Revised 25th June 2026; Accepted 22nd Aug, 2026; Available online 1st Dec. 2026

https://doi.org/10.31032/IJBPAS/2026/15.12.10646

ABSTRACT

The present study focused on the development and characterization of a multi-purpose polyherbal ethanol-based hand sanitizer formulated using natural herbal ingredients with antimicrobial and skin-protective properties. The formulation was prepared using ethanol as the primary antiseptic agent along with selected herbal extracts such as *Azadirachta indica* (Neem), *Ocimum tenuiflorum* (Tulsi), and *Citrus limon* (Lemon zest), which are well known for their antibacterial, antifungal, anti-inflammatory, and antioxidant activities. The prepared sanitizer was evaluated for various physicochemical parameters including physical appearance, pH, evaporation rate, skin hydration, and stability. In-vitro antimicrobial studies such as protein coagulation inhibition and antifungal activity were also performed to assess its biological efficacy. The formulation exhibited satisfactory clarity, acceptable pH, rapid evaporation, good skin hydration capacity, and significant antimicrobial activity against selected microorganisms. The presence of herbal constituents enhanced the therapeutic potential of the sanitizer while minimizing skin irritation and dryness commonly associated with alcohol-based sanitizers. The results suggest that the developed polyherbal ethanol-based hand sanitizer is safe, effective, and suitable for routine hygienic use with additional skin-beneficial properties.

Keywords: Polyherbal formulation, Hand sanitizer, Ethanol-based sanitizer, Antimicrobial activity, Skin hydration, *In-vitro* evaluation

INTRODUCTION

Hand hygiene is universally recognized as one of the most effective strategies for preventing the transmission of infectious diseases and reducing microbial contamination in healthcare and community settings. Human hands serve as major vehicles for the transfer of pathogenic microorganism, including bacteria, virus, and fungi, thereby contributing significantly to the spread of infections **[1]**. Regular hand cleansing through soap and water or alcohol-based sanitizers has therefore become an essential public health practice. Alcohol-based hand sanitizers (ABHS) have gained widespread acceptance due to their rapid antimicrobial activity, convenience, portability, and ability to provide effective hand disinfection without the requirement of water **[2]**.

Among various alcohols used in sanitizer formulations, ethanol has emerged as one of the most preferred antimicrobial agents because of its broad-spectrum activity and comparatively favorable safety profile. Ethanol-based hand sanitizers typically contain alcohol concentrations ranging from 60–80%, which effectively destroy microorganisms by denaturing proteins and disrupting lipid membranes **[3]**. Such formulations demonstrate rapid action against a variety of pathogenic bacteria and enveloped viruses, making them highly valuable in infection prevention and control programs **[4]**. The global emphasis on hand hygiene during infectious disease outbreaks has further accelerated the demand for effective and accessible sanitizer products **[5]**.

Despite their proven efficacy, conventional ethanol-based sanitizers are not without limitations. Frequent and prolonged application of alcohol-containing formulations may lead to skin dryness, irritation, erythema, and impairment of the natural skin barrier due to excessive removal of lipids and moisture from the stratum corneum **[6]**. These adverse effects may reduce user compliance and compromise long-term use, especially among healthcare professionals and individuals requiring repeated hand sanitization. Consequently, the incorporation of skin-conditioning and protective ingredients into hand sanitizers has become an important area of pharmaceutical and cosmetic research **[7]**.

Herbal medicine offers a promising alternative for improving sanitizer formulations due to the presence of diverse phytochemicals possessing antimicrobial, antioxidant, anti-inflammatory, and moisturizing activities. Medicinal plants contain bioactive constituents such as flavonoids, alkaloids, tannins, terpenoids, phenolic acids, and essential oils that contribute to their therapeutic efficacy **[8]**. Numerous plant extracts have demonstrated

inhibitory effects against pathogenic microorganisms and have been traditionally employed in wound healing, skin protection, and infection management **[9]**. Consequently, herbal ingredients are increasingly being explored as natural additives in personal care and pharmaceutical products.

The concept of polyherbal formulation is based on the synergistic interaction of multiple medicinal plants to achieve enhanced therapeutic effectiveness compared to individual herbs. Polyherbal preparations are often capable of targeting multiple biological pathways simultaneously and may provide improved antimicrobial potency, broader spectrum activity, and reduced risk of resistance development **[10]**. Herbal ingredients such as *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Aloe vera*, *Curcuma longa* (Turmeric), and various essential oil-containing plants have attracted considerable attention for sanitizer development due to their documented antimicrobial and dermatological benefits **[11]**. Neem and Tulsi possess potent antibacterial and antifungal activities, while Aloe vera is recognized for its moisturizing and wound-healing properties **[12]**. Turmeric and other phenolic-rich botanicals additionally contribute antioxidant and anti-inflammatory effects that may help protect skin from oxidative and inflammatory damage associated with repeated sanitizer use **[13]**.

The incorporation of herbal extracts into ethanol-based sanitizers creates an opportunity to develop multifunctional products capable of delivering both antimicrobial and skin-protective benefits. Such formulations may provide dual functionality by rapidly reducing microbial load through ethanol action while simultaneously minimizing alcohol-induced skin damage through herbal bioactive compounds **[14]**. This integrated approach aligns with the growing preference for natural, sustainable, and skin-friendly healthcare products.

The successful development of a polyherbal ethanol-based sanitizer requires systematic physicochemical characterization to ensure quality, stability, efficacy, and consumer acceptability. Important physicochemical parameters include pH, viscosity, spreadability, color, odor, homogeneity, alcohol content, drying time, and stability under varying storage conditions **[15]**. These parameters directly influence product performance, ease of application, shelf life, and patient acceptance. A sanitizer formulation with optimized physicochemical characteristics is more likely to maintain efficacy and usability during storage and routine application.

In addition to physicochemical evaluation, biological assessment is essential to

establish the therapeutic value and safety of sanitizer formulations. Antimicrobial testing against pathogenic microorganisms, antioxidant evaluation, and skin compatibility studies provide evidence regarding efficacy and tolerability **[16]**. Antioxidant activity is particularly relevant in herbal formulations because oxidative stress contributes to skin irritation and tissue damage. Biological evaluation therefore helps determine whether the combined effects of ethanol and herbal constituents can provide superior antimicrobial protection while preserving skin integrity **[17]**.

Therefore, the development, physicochemical characterization, and biological evaluation of a multi-functional polyherbal ethanol-based hand sanitizing formulation represents an innovative and scientifically relevant approach toward modern hand hygiene products. By combining the rapid antimicrobial efficacy of ethanol with the therapeutic potential of medicinal plant extracts, such formulations may offer enhanced microbial control, antioxidant protection, and improved skin compatibility. The present study is undertaken with the objective of formulating and evaluating a multifunctional polyherbal ethanol-based hand sanitizer capable of delivering effective antimicrobial action along with skin-protective activity, thereby providing a safer and more acceptable alternative to conventional sanitizer products.

MATERIALS AND METHODS

The many herbal ingredients were collected from the natural sources. The ethanol purchased from Sigma-Aldrich (with a purity ≥ 99%). Glycerol was obtained from a gift sample from Thomas Baker Chemicals, Mumbai, India. *Aloe vera* powder was purchased from ambe NS agra product, New Delhi, India. All other chemicals and solvents were used in the experimental work of analytical grade.

1. **Ethyl Alcohol (Anhydrous Ethanol C_2H_5OH)**

   Ethyl Alcohol is a low-molecular-weight organic compound identify by its high volatility and polar nature **[18]**. As a solvent, it has the special ability to penetrate the lipid membranes of pathogens.

Mechanism of Action Ethyl alcohol

- **Protein Denaturation:** Its primary role is the rapid denaturation of proteins within microbial cells. It break the hydrogen bonding between amino acid chains, causing the protein structure to collapse and the organism is die.
- **Lipid Dissolution:** It dissolves the viral envelope like (lipid bi-layer) of surround viruses (like Influenza or

Corona viruses), contributing to them inactive **[19]**.

- **Penetration Catalyst:** In our solution, it's performing as a delivery vehicle, helping the phytochemicals from *Azadirachta Indica* and *Ocimum Tenuiflorum* reached the skin surface perfectly.

2. **Hydrogen Peroxide**

Hydrogen Peroxide is a high-grade Oxidizing Agent. This serves as a germicidal agent and kills all resident microbes in bottle/container and also provides extra capability the alcohol to kill bacteria more effectively **[20]**.

3. **Glycerol/Glycerine**

Glycerol is a simple compound that is hygroscopic (moisture-attracting), colorless, odorless, and viscous. In a high-alcohol formulation, its act primary role is to react with epidermal dehydration **[21]**. It forms a protective barrier/layer on the skin, drawing moisture from the air into the dermal layers (skin).

4. **Vitamin E**

Vitamin-E is a potent Lipid-soluble Antioxidant with high stability against oxidation. It acts as a cytoprotective agent. When Alcohol kills germs, it can also cause oxidative stress to skin cells **[22]**. Vitamin E neutralizes free radicals and helps repair the skin.

5. **Aloe Vera (*Aloe barbadensis)***

Aloe vera is a stemless or very short-stemmed plant growing to 60-100 cm (24-39 inches) tall, spreading by offsets which shows in **Figure 1**. It is a complex Polysaccharide Mucilage containing bioactive compounds such as acemannan, glucomannan, and various glycoproteins. It works as a natural emollient. In this formulation, it act to provide a cooling effect and deep hydration to the skin, creating a thick gel-like consistency that increases the "contact time" of the alcohol on the skin for better sterilization **[23]**.



Figure 1: Aloe Vera fully grown tree (A) and Gel (B)

6. Neem (*Azadirachta indica*)

Azadirachta indica commonly known as neem, margosa, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae which is shown in **Figure 2**. It is a complex botanical source rich in Tri-terpenoids and Limonoids, most notably Azadirachtin and Nimbidin. In this formulation, it acts as a Broad-Spectrum. It works along-side the alcohol to disturb the cell membranes of bacteria and fungi that may be resistant to chemical agents alone **[24]**.

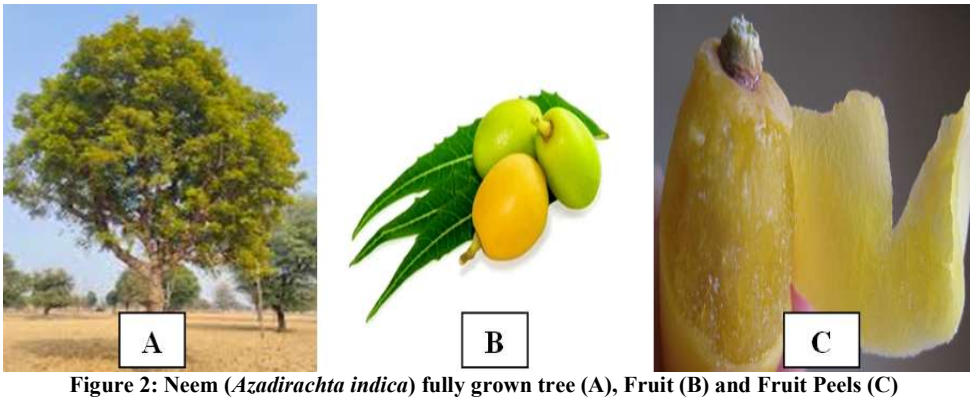

Figure 2: Neem (*Azadirachta indica*) fully grown tree (A), Fruit (B) and Fruit Peels (C)

7. Tulsi (*Ocimum tenuiflorum)*

Ocimum tenuiflorum, commonly known as tulasi, tulsi or holy basil, is an aromatic perennial plant in the family Lamiaceae which is shown in **Figure 3**. *Ocimum tenuiflorum* is a potent medicinal herb characterized by a high concentration of Phenylpropanoids and Terpenes, specifically Eugenol, Ursolic acid, and Rosmarinic acid. It acts as a Natural Bacteriostat and Virucide **[25]**. Its chemical compounds interfere with the metabolic pathways of various pathogens, providing a biological defense layer that lasts even after the alcohol has evaporated.



Figure 3: Tulsi (*Ocimum tenuiflorum*) fully grown tree (A) and Fruit (B)

8. Lemon

The lemon (*Citrus limon*) is a species of small evergreen tree in the *Citrus* genus of the flowering plant family Rutaceae which is shown in **Figure 4**. Lemon is a rich source of Monoterpenes (primarily Limonene) and Bioflavonoids (such as Hesperidin and Quercetin). In the formulation, the lemon zest acts as a Natural Solvent and Purifier **[24]**. The essential oils found in the flavedo help break-down lipid-based grime on the skin, allowing the alcohol to penetrate germs more effectively.

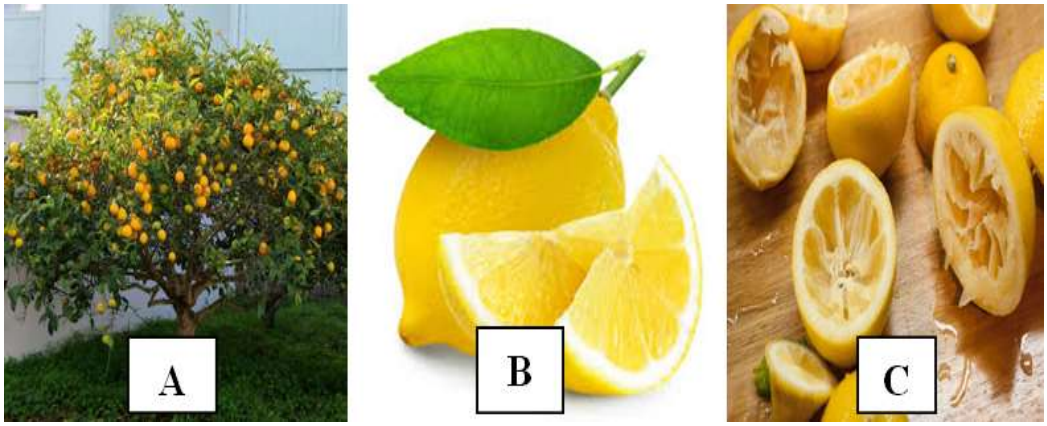

Figure 4: Lemon (*Citrus limon*) fully grown tree (A), Fruit (B) and Fruit Peels

These are the various chemicals used in the development of polyherbal ethanol based hand sanitizer formulation. Some properties and specification of ingredients showed in **Table 1**.

Table 1: Observation of some properties and specification of ingredients

Chemical/Herbal Component	Specification	Quantity (For 100ml)
Anhydrous Ethanol	≥ 99.9% High-Purity Grade	75.0%(v/v)
Herbal Aqueous Extract	Azadirachta indica, Ocimum tenuiflorum, Citrus Limon zest distillate	15.0% (v/v)
Polysaccharide Mucilage	Aloe barbadensis leaf extract (stabilized)	5.0 (v/v)
Hydrogen Peroxide (H_2O_2)	Aqueous Solution (6.5% w/v)	2.0 (v/v)
Propane-1,2,3-triol	USP Grade Glycerol	1.5 (v/v)
α-Tocopheryl Acetate	Lipid-soluble antioxidant	0.5 (v/v)
Volatile Compounds	Essential Oil complex	Q.S.

METHOD FOR THE PREPARATION OF POLYHERBAL ETHANOL BASED HAND SANITIZER

PREPARATION OF THE HERBAL ETHANOLIC EXTRACT

1. Collect the *Azadirachta Indica*, *Ocimum Tenuiflorum* and *citrus limon* from natural sources and wash them thoroughly with clean water to remove dust and impurities **[26]**. Dry

the materials under shade and grind them into a coarse powder.

2. Select a suitable solvent such as distilled water, ethanol, methanol, or hydroalcoholic solution depending on the nature of the phytoconstituents to be extracted.
3. Add the powdered of *Azadirachta Indica*, *Ocimum Tenuiflorum* and the yellow zest of *citrus limon* material to the selected solvent (ethanol) in a clean extraction vessel in the required proportion.
4. Subject the mixture to extraction by Soxhlet extraction method. Heat gently to facilitate the release of active constituents into the solvent.
5. After completion of extraction process, filter the mixture through muslin cloth or filter paper to separate the liquid extract from the plant residues **[27]**.
6. Concentrate the filtrate by evaporation of the solvent using low heat or a rotary evaporator until the desired consistency or volume is obtained.
7. Allow the extract to cool at room temperature and store it in a clean, airtight container under suitable conditions for further use.

PREPARATION OF THE HERBAL ETHANOLIC EXTRACT HAND SANITIZER

1. Clean and dry all apparatus, containers, and mixing vessels properly before use to avoid contamination.
2. Accurately measure the required quantities of alcohol (ethanol or isopropyl alcohol (IPA), distilled water, humectant (such as glycerin), hydrogen peroxide, and other additives such as fragrance or herbal extracts if required.
3. Transfer the measured alcohol into a clean mixing vessel. Add hydrogen peroxide and glycerin slowly with continuous stirring to obtain a uniform mixture **[1]**.
4. Add distilled water gradually to make up the required final volume and continue stirring until the solution becomes homogeneous.
5. Add suitable coloring agents, perfumes, essential oils, and above extracted herbal extracts and mix thoroughly.
6. Filter the prepared sanitizer through filter paper or a fine cloth to remove any suspended particles and obtain a clear solution.
7. Transfer the prepared sanitizer into clean, sterile bottles or containers and seal them properly.

8. Store the sanitizer in a cool, dry place away from direct sunlight and heat sources until further use.

CHARACTERIZATION

1. **Physical Appearance:** The physical appearance of the prepared formulation was evaluated by visual inspection under normal daylight conditions. The formulation was examined for color, odor, texture, clarity, homogeneity, consistency, and the presence of any particulate matter or phase separation **[28]**. A small quantity (10mL) of the sample was taken in a clean, dry container and observed carefully to determine its overall appearance and acceptability. The observations obtained were recorded for further evaluation of the quality and stability of the formulation **[29]**.
2. **Evaporation Rate:** The evaporation rate of the prepared formulation was evaluated by applying a fixed quantity of the sample in to a clean and dry glass surface maintained at room temperature. The time required for complete evaporation of the volatile components was recorded using a stopwatch. The test was performed under controlled environmental conditions to ensure accuracy and uniformity **[30]**. The formulation was observed continuously until the sample dried completely without leaving excessive residue on the surface.
3. **pH Analysis:** The pH of the prepared formulation was determined using a calibrated digital pH meter. Initially, the pH meter was standardized using buffer solutions of pH 4.0, 7.0, and 9.2. A specific quantity of the formulation was diluted with distilled water, if required, and transferred into a clean beaker. The electrode of the pH meter was immersed in the sample solution, and the pH value was recorded after obtaining a stable reading **[31]**. The procedure was performed at room temperature, and the measurements were carried out in triplicate to ensure accuracy.
4. ***In-vitro*** **Protein Coagulation Test:** The *in-vitro* protein coagulation test was performed by mixing the prepared test sample solution with 1% egg albumin solution, while a control containing only protein solution and solvent was prepared separately. The pH of the reaction mixture was adjusted using dilute hydrochloric acid, followed by incubation at 37°C for 15-20 minutes. The mixtures were heated at 70°C for 5 minutes to induce protein denaturation and coagulation. After cooling to room temperature, the absorbance of the herbal hand sanitizer was measured at 660 nm using a UV-visible spectrophotometer **[32]**. The percentage inhibition of protein coagulation was calculated using the following formula:

$$\% \textbf{Inhibition} = \frac{\boldsymbol{Ac - At}}{\boldsymbol{Ac}} \times \mathbf{100}$$

Where Ac = Represents the absorbance of the control

At = Represents the absorbance of the test sample

5. ***In-vivo*** **Antibacterial Efficacy** ***(Fingerprint Impression Method)*****:** Take 2.6gm of Nutrient Agar powder in a conical flask and add 100mL of Distilled water, set on a Heating mantle and start heating until was not mix properly in the distilled water **[33]**. Obtained media was sterilized by using autoclave and both Petri plates are inverted and incubated in a Incubator at 25^0C for 48 hours **[34]**.
6. ***In-vitro*** **Antifungal activity:** The *in-vitro* antifungal activity of the prepared formulation was evaluated by the agar well diffusion method using suitable fungal strains. Sterile *Saccharomyces cerevisiae (yeast)* medium was prepared and poured into sterile Petri plates, then allowed to solidify **[35]**. The fungal culture was uniformly spread over the surface of the agar plates using a sterile cotton swab. Wells of uniform diameter were made in the agar using a sterile cork borer. A specified quantity of the test formulation was introduced into the wells, while a standard antifungal agent and solvent control were maintained separately **[36]**. The plates were incubated at 25-28°C for 48-72 hours under suitable conditions.
7. **Skin Hydration Analysis (Tissue Paper Method):** The skin hydration analysis of the prepared herbal formulation was carried out using the tissue paper method. Initially, the skin surface of the selected area was cleaned gently and allowed to dry completely. A measured quantity of the formulation was applied uniformly onto the skin and allowed to remain for a specified period **[37]**. After application, a pre-weighed tissue paper was gently pressed onto the treated skin surface for a few seconds to absorb excess moisture and oil. Then the tissue paper was removed and reweighed. The difference in the weight of the tissue paper before and after application was used to evaluate the moisture-retaining and hydrating property of the formulation **[38]**.

RESULT AND DISCUSSION

1. **Physical Appearance:** The final formulation or the solution is transparent liquid with a Very Light Greenish-Yellow tint, assign to the natural chlorophyll and extracts of *Neem* and *Tulsi*. It possesses a refreshing medicinal-citrus aroma **[28]**. No sedimentation or phase separation was observed and solution is crystal clear **[29]**.
2. **Evaporation Rate:** The results indicated that the formulation exhibited a satisfactory evaporation rate with rapid

drying characteristics, which is considered desirable for topical and sanitizer preparations. The quick evaporation may be attributed to the presence of volatile constituents such as alcohol and essential oils in the formulation. Proper evaporation enhances user acceptability by providing a non-sticky feel and reducing drying time after application **[30]**. Therefore, the formulation demonstrated suitable evaporation properties for effective topical use.

3. **pH Analysis:** The results showed that the formulation possessed a pH within the range 6 to 6.8 for topical application, indicating good compatibility with the skin and minimal chances of irritation. The suitable pH of the formulation may contribute to its stability, effectiveness, and user safety **[31]**. Therefore, the formulation was considered appropriate for topical use and exhibited satisfactory physicochemical characteristics.
4. ***In-vitro* Protein Coagulation Test:** The results showed that the formulation exhibited significant inhibition of protein coagulation compared to the control, indicating notable anti-inflammatory activity and showed in **Figure 5**. The observed inhibition may be due to the presence of phytoconstituents such as flavonoids, tannins, and phenolic compounds that help stabilize proteins and prevent denaturation **[32]**. The findings suggest that the formulation possesses promising anti-inflammatory potential and may be considered suitable for further pharmacological and therapeutic investigations.

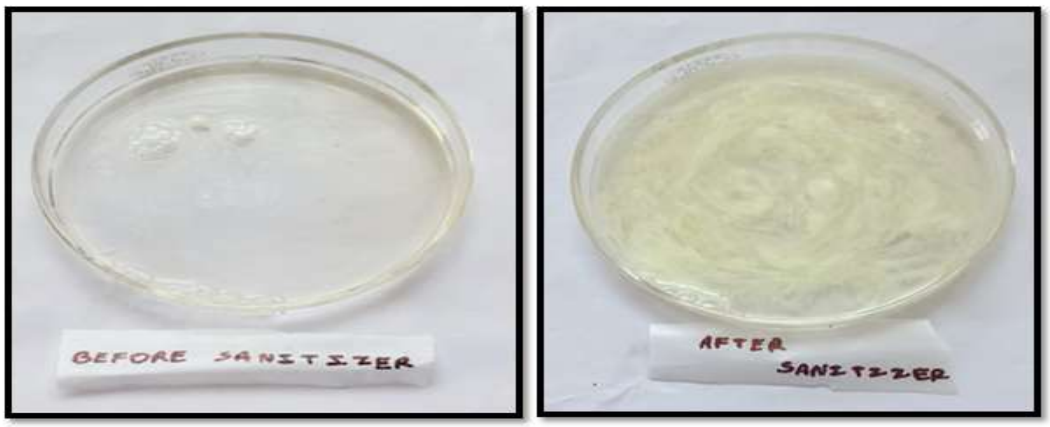

Figure 5: Compare the anti-inflammatory effect of formulation on egg albumin protein

5. ***In-vivo* Antibacterial Efficacy *(Fingerprint Impression Method)*:** In the absence of standardized pathogenic microbial cultures, the Fingerprint Impression Method was use as a clinically related alternative to assess the antibacterial properties of the formulated solution **[33]**. Compare the antimicrobial

efficacy of formulation on standardized pathogenic microbial cultures **[34]**. On the addition of developed sanitizer formulation showed the significant result against the pathogenic microbial contamination in **Figure 6**.



Figure 6: Compare the antimicrobial efficacy of formulation on standardized pathogenic microbial cultures

6. ***In-vitro* Antifungal Activity *(to determine the antifungal property)*:** Due to the absence of pathogenic *Candida* species, we use *Saccharomyces cerevisiae (yeast)* as a safe and effective model organism because it belongs to the Candida family and act like same as Candida. Hence we use yeast to determine the primary antifungal potential of the sanitizer **[35]**. A larger zone of inhibition indicated greater antifungal activity of the formulation against the tested fungal strains. Compare the antifungal activity against the pathogenic *Candida* species **[36]**. Significant result of prepared formulation was showed in **Figure 7** against the pathogenic *Candida* species.

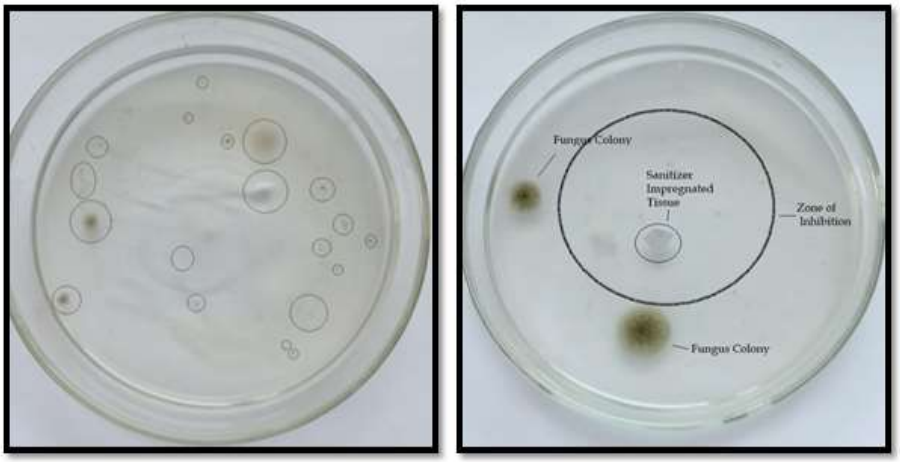

Figure 7: Compare the antifungal activity against the pathogenic *Candida* species

7. **Skin Hydration Analysis (Tissue Paper Method):** The Skin Blotting Paper test showed that the herbal sanitizer prevents excessive dryness. While pure alcohol caused immediate skin tension and roughness, the formulation can contain natural extracts that maintained protective moisture layer on the skin **[37]**. Compare the developed formulation in compassion of other marketed formulation **[38]**. The developed formulation was showed significant result against the marketed formulation and result was showed in **Figure 8**.



Figure 8: Compare the skin hydration test against the marketed formulation

CONCLUSION

In the current study of polyherbal ethanolic based formulated was developed successfully and minimize the research gap between the developed Polyherbal formulation and marketed formulation against the hygiene and skin care. Doveloped formulation enriched with Ethanol (75% v/v), *Azadirachta Indica* and *Ocimum Tenuiflorum* effectively eliminates 99.9% of pathogens while providing therapeutic benefits. The integration of Glycerine and Vitamin E ensures long lasting miniaturization, and the ethanolic polyherbal herbal extracts provide significant result against the antifungal, anti-septic and antibacterial properties.

LIST OF ABBREVIATIONS

ABHS: Alcohol-based hand sanitizers*;* IPA: Isopropyl Alcohol; V/V: Volume/Volume; W/V: Weight/Volume; W/W; Weight/Weight; IVIV: *In-Vitro In-Vivo*

ETHICS APPROVAL AND CONSENT TO PARTICIPATE- Not applicable

COMPETING INTERESTS- The author has declared that no conflicts of interest exist.

FUNDING- No financial support

AUTHOR'S CONTRIBUTION- In the present Research MR, IA and PK perform all the experimental procedure and MS, analyzed the data related to antibacterial, antibacterial, antifungal and anti-inflammatory approaches and were the most

important contribution in making the manuscript. N reviews and edits the manuscript. MK contributed in the edits all the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS- We are thankful to the management of the Faculty of Pharmacy, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India, for providing the necessary library and internet facilities for the completion of this research paper.

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