RESEARCH PAPER

Formulation and Physiochemical Characterization of a Herbal Surface Cleaner Based on Neem and Lemon Herbal Actives

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Abstract

The increasing awareness of the detrimental health and environmental effects of synthetic chemical-based cleaning products has catalyzed research into natural, biodegradable alternatives. This study focuses on the formulation and comprehensive physiochemical characterization of a herbal surface cleaner utilizing neem (Azadirachta indica) oil and lemon (Citrus limon) oil as primary active ingredients. Five formulations (A-E) were prepared with varying concentrations of neem and lemon extracts ranging from 3% to 7% each, using purified water as the base. The formulations were evaluated for physical parameters including appearance, color, odor, pH, grittiness, and staining potential on white tiles. Chemical characterization involved foam testing for saponin content and color testing for triterpenoid identification. Microbiological safety was assessed through bioburden testing for total bacterial count (TBC) and total fungal count (TFC). Results demonstrated concentration-dependent properties: formulations up to 5% maintained free-flowing consistency, smooth texture, and no staining, while 6-7% formulations exhibited slight staining. All formulations showed acidic pH (3.5-3.8) favorable for antimicrobial activity. Foam tests confirmed stable foam formation (>5 minutes) across all concentrations, indicating saponin presence. Color tests revealed progressive intensification from pale green to dark blue green with increasing concentration, confirming triterpenoid content. Bioburden testing showed microbial counts below 10 CFU/ml for all formulations, meeting safety standards. Formulation C (5% neem + 5% lemon) emerged as optimal, balancing desirable physical properties with confirmed phytochemical content and excellent microbiological safety. This research establishes that neem and lemon-based herbal formulations offer promising potential as effective, safe, and environmentally sustainable alternatives to chemical-based surface cleaners.

Keywords: Herbal surface cleaner, Azadirachta indica, Citrus limon, physiochemical characterization, saponins, triterpenoids, bioburden, green chemistry

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1. Introduction

1.1 Background and Rationale

The global cleaning products industry has experienced a significant paradigm shift in recent years, driven by escalating consumer awareness regarding the potential health hazards associated with synthetic chemical cleaners and their detrimental impact on environmental ecosystems. Conventional surface cleaners, which dominate household and institutional markets, typically contain harsh chemicals including chlorine compounds, ammonia, phosphates, phthalates, and synthetic fragrances that pose significant risks to human health and ecological balance [1]. Prolonged exposure to these chemicals has been linked to respiratory disorders, dermatological conditions, endocrine disruption, and potential carcinogenic effects [2].

The environmental footprint of synthetic cleaners extends beyond direct human exposure. Upon disposal, these chemical formulations enter water systems, where they persist due to their non-biodegradable nature, causing eutrophication, aquatic toxicity, and disruption of fragile microbial ecosystems in wastewater treatment facilities [3]. The cumulative environmental burden has prompted regulatory bodies worldwide to impose stricter guidelines on chemical formulations and encourage the development of green alternatives [4].

In response to these concerns, herbal surface cleaners have emerged as viable alternatives, formulated from natural plant extracts that demonstrate antimicrobial efficacy while maintaining biodegradability and environmental compatibility [5]. These plant-based formulations leverage the inherent bioactive compounds present in medicinal and aromatic plants, which have evolved over millions of years as part of plants' defense mechanisms against pathogenic microorganisms [6].

1.2 Neem (Azadirachta indica): Phytochemistry and Antimicrobial Properties

Among the vast repository of medicinal plants, neem (Azadirachta indica) occupies a position of exceptional

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significance, particularly in the Indian subcontinent where it has been utilized for millennia in traditional medicine, agriculture, and household applications. Often referred to as "the village pharmacy," neem has been integral to Ayurvedic, Unani, and Siddha medical systems [7].

The therapeutic potential of neem stems from its complex phytochemical profile, comprising over 140 biologically active compounds. The most extensively studied among these are azadirachtin, nimbin, nimbidin, nimbolide, and gedunin—all belonging to the triterpenoid class of compounds [8]. These phytochemicals demonstrate remarkable antimicrobial activity through multiple mechanisms including membrane disruption, enzyme inhibition, and biofilm prevention [9]. Neem extracts have demonstrated efficacy against a wide range of microorganisms including bacteria, fungi, and viruses [10].

Recent studies by Patankar and Chandak (2018) demonstrated that neem-based sanitizer formulations showed significant antimicrobial activity against both standard cultures and hand isolates, including antibiotic-resistant strains, with zones of inhibition comparable to or exceeding commercial alcohol-based sanitizers [11].

1.3 Lemon (Citrus limon): Bioactive Components and Cleaning Efficacy

Lemon (Citrus limon), while widely recognized for its refreshing fragrance and culinary applications, possesses sophisticated antimicrobial chemistry that makes it invaluable in herbal cleaning formulations. The bioactive potential of lemon resides primarily in two components: the essential oil extracted from the peel and the organic acid-rich juice [12].

Lemon essential oil contains high concentrations of monoterpenes, particularly d-limonene (constituting 70-90% of the oil), along with β-pinene, γ-terpinene, and citral [13]. These compounds exhibit antimicrobial activity through disruption of microbial membrane integrity, interference with cellular respiration, and inhibition of quorum sensing [14].

Research demonstrated that citrus oil concentrations as low as 0.3-0.5% effectively inhibited Escherichia coli growth, while Salmonella species showed susceptibility at even lower concentrations (0.15-0.3%) [15]. Additionally, lemon juice contains citric acid, which acts as a chelating agent, binding metal ions essential for microbial enzyme function while creating acidic environments unfavorable for microbial proliferation [16].

1.4 Synergistic Interactions and Research Rationale

The combination of neem and lemon in cleaning formulations offers potential synergistic effects that enhance overall efficacy beyond what either ingredient achieves independently [17]. Several mechanisms underlie this synergy: complementary antimicrobial targets, enhanced solubility of hydrophobic compounds, and combined surfactant-antimicrobial action [18].

Despite growing interest in herbal cleaners and established knowledge regarding individual antimicrobial properties of neem and lemon, systematic studies on combined formulations with controlled concentration gradients remain limited. Critical parameters requiring investigation include optimal concentration ranges balancing efficacy with physical stability, concentration-dependent effects on physical properties, and standardized evaluation protocols for herbal cleaner performance [19].

The present study addresses these gaps by formulating five distinct concentrations of neem and lemon combination (3% to 7% each) and subjecting them to comprehensive physical, chemical, and microbiological evaluation.

1.5 Objectives

The primary objectives of this research are:

1. To formulate herbal surface cleaners with varying concentrations (3-7%) of neem oil and lemon oil in combination
2. To evaluate physical characteristics including appearance, color, odor, pH, grittiness, and staining potential
3. To assess chemical properties through foam testing (saponin presence) and color testing (triterpenoid identification)
4. To determine microbiological safety via bioburden testing for total bacterial and fungal counts
5. To identify the optimal concentration range balancing desirable physical properties with chemical efficacy

2. Materials and Methods

2.1 Raw Materials

- **Neem Oil:** Water-soluble neem oil (Azadirachta indica) procured from certified herbal extract supplier.
- **Lemon Oil:** Water-soluble lemon oil (Citrus limon) obtained from standardized commercial source.
- **Purified Water:** Freshly prepared purified water meeting pharmacopoeial standards

2.2 Equipment

- **Magnetic Stirrer:** REMI 2MLH magnetic stirrer with Teflon-coated stirring bars
- **pH Meter:** Mettler Toledo digital pH meter calibrated with standard buffer solutions.
- **BOD Incubator:** Thermolab for microbiological incubations
- **Laminar Air Flow:** Clean Air Instruments horizontal laminar airflow cabinet with HEPA filtration

2.3 Microbiological Media

- **Soyabean Casein Digest Agar (SCDA):** HiMedia Laboratories for total bacterial count
- **Sabouraud Dextrose Agar with Chloramphenicol (SDA-C):** HiMedia Laboratories for total fungal count

2.4 Formulation Development

Five formulations were prepared with equal proportions of neem and lemon oils as shown in Table 1.

Table 1. Composition of Neem and Lemon Based Herbal Surface Cleaner Formulations

Formulation Code	Neem Oil (% w/w)	Lemon Oil (% w/w)	Purified Water (% w/w)
A	3.0	3.0	94.0
B	4.0	4.0	92.0
C	5.0	5.0	90.0
D	6.0	6.0	88.0
E	7.0	7.0	86.0

Preparation Method: For each formulation, measured quantities of purified water were transferred to clean glass beakers and placed on magnetic stirrers at moderate speed (300-500 rpm). Neem oil was added slowly to the vortex, followed by lemon oil. Mixing continued for 15-20 minutes until visually homogeneous. Three independent batches (T1, T2, T3) of each formulation were prepared on different days to assess reproducibility.

2.5 Physical Evaluation Parameters

Appearance: Approximately 50 ml of each formulation was observed under adequate illumination against white and black backgrounds. Flow characteristics and presence of particulate matter were recorded.

Color: A drop of formulation (approximately 0.5 ml) was placed on a clean white ceramic tile surface and observed immediately.

Odor: Evaluated samples presented in random order, recording odor descriptors and intensity.

pH: The pH meter was calibrated daily. For each formulation, 20 ml sample was taken, and pH recorded after stabilization at 25°C ± 2°C. Three measurements were performed for each batch.

Grittiness: A small drop of formulation (approximately 0.2 ml) was rubbed between thumb and index finger for 10-15 seconds to detect any particles.

Staining: White ceramic tiles were cleaned, and 5 ml of formulation was applied with microfiber cloth, allowed to dry, and examined for residues or discoloration.

2.6 Chemical Evaluation

Foam Test (Saponin Detection): From each formulation, 1 ml was diluted with 5 ml distilled water in a test tube and shaken vigorously for 60 seconds. Foam height was measured immediately, and stability was assessed by observing time for foam collapse to half original height.

Color Test (Triterpenoid Detection): To 2 ml of formulation, 1 drop of 10% copper sulfate ($CuSO_4$) was added, followed by 1 ml concentrated sulfuric acid (H_2SO_4) added carefully along the test tube wall. The mixture was heated gently in a boiling water bath for 2-3 minutes, cooled, and color development was observed.

2.7 Microbiological Evaluation

Sample Preparation: Under aseptic conditions in laminar airflow, 1 ml of formulation was transferred to sterile petri plates in duplicate. Approximately 15-20 ml of sterile molten SCDA (45°C ± 2°C) was poured for bacterial count, and SDA-C for fungal count. Plates were rotated for uniform mixing and allowed to solidify.

Incubation: SCDA plates were incubated at 30-35°C for 48-72 hours; SDA-C plates were incubated at 20-25°C for 5 days in BOD incubators.

Negative Control: Sterile neutralizing medium was processed identically to detect contamination during testing.

3. Results and Discussion

3.1 Physical Evaluation Results

Table 2. Appearance, Color, and Odor Characteristics

Concentration	Trial	Appearance	Color	Odor
3%	T1-T3	Free flowing	Creamy	Fresh, Citrus Neem
4%	T1-T3	Free flowing	Creamy	Fresh, Citrus Neem
5%	T1-T3	Free flowing	Creamy	Balanced neem and lemon

Concentration	Trial	Appearance	Color	Odor
6%	T1-T3	Free flowing	Creamy	Balanced neem and lemon
7%	T1-T3	Slightly Viscous	Creamy	Balanced neem and lemon

Formulations containing 3%, 4%, 5%, and 6% concentrations consistently exhibited free-flowing liquid characteristics across all three trials. At 7% concentration, a transition to "slightly viscous" consistency was observed uniformly. All formulations exhibited consistent creamy coloration across all concentrations and trials. A concentration-dependent shift in odor characteristics was observed. At lower concentrations (3-4%), panelists described the odor as "Fresh, Citrus Neem." At 5%, 6%, and 7% concentrations, odor characterization shifted to "Balanced neem and lemon."

Table 3. pH, Grittiness, and Staining Characteristics

Concentration	Trial	pH	Grittiness	Staining (White Tile)
3%	T1	3.8	Smooth	No Stain
	T2	3.8	Smooth	No Stain
	T3	3.7	Smooth	No Stain
4%	T1	3.6	Smooth	No Stain
	T2	3.7	Smooth	No Stain
	T3	3.6	Smooth	No Stain
5%	T1	3.6	Smooth	No Stain
	T2	3.6	Smooth	No Stain
	T3	3.5	Smooth	No Stain
6%	T1	3.5	Smooth	Faded
	T2	3.5	Smooth	Faded
	T3	3.5	Smooth	Faded
7%	T1	3.5	Smooth	Faded
	T2	3.5	Smooth	Faded
	T3	3.5	Smooth	Faded

All formulations exhibited acidic pH values ranging from 3.5 to 3.8. A slight concentration-dependent decrease in pH was observed: 3% formulations averaged pH 3.77, 4% averaged pH 3.63, while 5%, 6%, and 7% formulations stabilized at pH 3.5-3.6.
All formulations across all concentrations and trials received "Smooth" ratings for grittiness, indicating complete solubilization of active ingredients.
A concentration-dependent staining pattern emerged: formulations containing 3%, 4%, and 5% concentrations consistently showed "No Stain" on white ceramic tiles. At 6% and 7% concentrations, all trials exhibited "Faded" staining.

3.2 Chemical Test Results

Table 4. Foam and Color Test Characteristics

Concentration	Trial	Foam Height	Foam Stability (min)	Color Test (Triterpenoids)
3%	T1-T3	Approx. 2.0 cm	>5	Pale Green
4%	T1-T3	Approx. 2.0 cm	>5	Light Blue Green
5%	T1-T3	Approx. 2.0 cm	>5	Blue Green
6%	T1-T3	Approx. 2.0 cm	>5	Dark Blue Green
7%	T1-T3	Approx. 2.0 cm	>5	Dark Blue Green

All formulations produced approximately 2.0 cm of foam upon standardized shaking, with foam stability exceeding 5 minutes in all cases, confirming the presence of saponins.
The color test revealed a clear concentration-dependent intensification pattern:
- **3%**: Pale green
- **4%**: Light blue green
- **5%**: Blue green
- **6-7%**: Dark blue green

3.3 Bioburden Test Results

Table 5. Bioburden Analysis

Concentration	Trial No.	TBC (CFU/ml)	TFC (CFU/ml)
3%	01-03	<10	<10
4%	01-03	<10	<10
5%	01-03	<10	<10
6%	01-03	<10	<10
7%	01-03	<10	<10

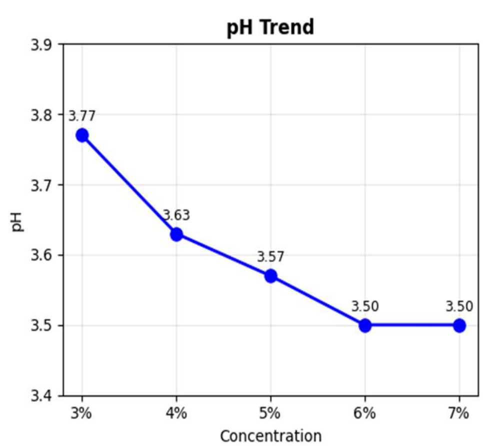
All formulations across all concentrations and trials exhibited total bacterial counts and total fungal counts below 10 CFU/ml—the detection limit of the method—indicating excellent antimicrobial activity and manufacturing hygiene.

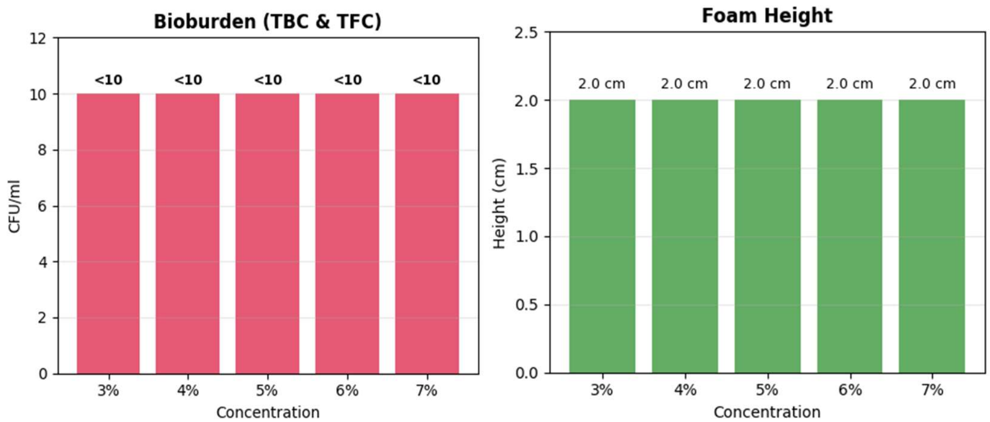
3.4 Summary of Concentration-Dependent Trends

Table 6. Summary of Concentration-Dependent Trends

Parameter	**3%**	**4%**	**5%**	**6%**	**7%**
Appearance	Free flowing	Free flowing	Free flowing	Free flowing	Slightly viscous
Color	Creamy	Creamy	Creamy	Creamy	Creamy
Odor	Fresh, Citrus Neem	Fresh, Citrus Neem	Balanced	Balanced	Balanced
pH	3.7-3.8	3.6-3.7	3.5-3.6	3.5	3.5
Grittiness	Smooth	Smooth	Smooth	Smooth	Smooth
Staining	None	None	None	Faded	Faded
Color Test	Pale Green	Light Blue Green	Blue Green	Dark Blue Green	Dark Blue Green
TBC/TFC (CFU/ml)	<10	<10	<10	<10	<10

The data reveal that 5% concentration represents an optimal balance point where free-flowing appearance is maintained, no staining is observed, balanced odor profile is achieved, good triterpenoid content is present, and excellent microbiological safety is maintained.





3.5 DISCUSSION

3.5.1 Physical Characteristics and Formulation Stability

The transition from free-flowing liquid at concentrations up to 6% to slightly viscous consistency at 7% represents an important physical boundary in this formulation system. This rheological shift likely results from increased oil droplet concentration approaching the maximum packing fraction, beyond which droplet-droplet interactions become significant, increasing viscosity [20]. In oil-in-water dispersions, viscosity depends on volume fraction of dispersed phase, droplet size distribution, interfacial properties, and continuous phase viscosity [21].

The observation that 6% formulations remain free flowing while 7% formulations become slightly viscous suggests that 6-7% represents the transition zone where these interactions become significant. For practical applications, both free-flowing and slightly viscous formulations remain usable, though dispensing characteristics may differ. Free-flowing formulations (3-6%) are ideal for spray bottles, while slightly viscous formulations (7%) may require squeeze bottles or pump dispensers [22].

The acidic pH range (3.5-3.8) observed across all formulations reflects the combined contributions of lemon oil (citric acid, ascorbic acid) and neem components. This pH range offers several functional advantages including enhanced antimicrobial activity, aid in saponification of fatty soils, and dissolution of alkaline mineral deposits [23]. Simon Jameson's patent on non-toxic antimicrobial compositions specifically claims compositions with pH adjusted to 2.0-4.0 for rapid bioburden reduction, noting that acidic conditions enhance the antimicrobial activity of plant oils [24].

The appearance of faded staining at 6-7% concentrations, while absent at lower concentrations, likely results from non-volatile residue remaining after water evaporation, terpenoid oxidation forming colored compounds, or film formation altering light reflection [25]. For stain-sensitive applications (e.g., glassware, glossy surfaces), the 3-5% concentration range would be preferred.

3.5.2 Phytochemical Confirmation and Functional Implications

The consistent foam formation (>2 cm, >5 min stability) across all concentrations confirms the presence of saponins with excellent surface-active properties. Saponins function as natural surfactants through surface tension reduction, emulsification, and enhanced detergency [26]. Beyond surfactant properties, saponins contribute antimicrobial activity through membrane disruption—their amphiphilic nature enables insertion into microbial membranes, causing permeability changes and cell lysis [27].

The concentration-dependent intensification of the color test (pale green → dark blue green) provides semi-quantitative confirmation of increasing triterpenoid content. Neem's triterpenoids, particularly the limonoids azadirachtin, nimbin, and salannin, are well-documented antimicrobial agents [28]. Mechanisms of triterpenoid antimicrobial activity include membrane disruption, enzyme inhibition, quorum quenching, and metal chelation [29].

The presence of triterpenoids in all formulations, confirmed by color testing, explains the excellent bioburden results and suggests potential for significant antimicrobial activity against surface pathogens. This aligns with findings by Patankar and Chandak (2018) who demonstrated that neem sanitizer exhibited zones of inhibition comparable to or exceeding commercial alcohol-based sanitizers [11].

3.5.3 Microbiological Safety and Self-Preservation

The absence of detectable bioburden (<10 CFU/ml) across all formulations and trials represents a significant finding with multiple implications. Unlike many herbal formulations that require added preservatives to prevent microbial growth, the neem-lemon combination appears inherently self-preserving [30]. This property aligns with consumer preferences for preservative-free natural products and simplifies formulation.

The results suggest broad-spectrum antimicrobial activity encompassing both bacteria and fungi, likely resulting from multiple active compounds with different mechanisms, reducing the probability of resistant organisms emerging [31]. The consistency across three independent trials indicates that the antimicrobial activity is robust to normal manufacturing variations.

Bioburden levels below 10 CFU/ml meet or exceed most regulatory requirements for non-sterile products, positioning these formulations favorably for commercial development [32]. These results compare favorably with microbiological specifications for commercial surface cleaners and disinfectants, which typically require bacterial counts below 100-1000 CFU/ml depending on regulatory jurisdiction [33].

3.5.4 Comparison with Reported Formulations

Natural cleaning agents using lemon peel extract, coconut shell, and eggshell, finding that lemon-only formulations exhibited pH 3.88 (similar to our 3% formulation at pH 3.7-3.8) with acceptable cleaning performance [5]. Their lemon-coconut shell formulation demonstrated prolonged froth stability, exceeding the >5 minutes stability observed in our study—though direct comparison requires considering different measurement methodologies.

The CSIR-Institute of Minerals and Materials Technology developed a natural disinfectant surface cleaner formulation utilizing neem or lemongrass in combination with soap nuts (Sapindus mukorossi), alum, and camphor [1]. Both formulations leverage neem's antimicrobial properties and aim for natural, non-toxic alternatives to chemical cleaners, though our formulation relies on lemon oil for acidity, fragrance, and additional antimicrobial activity.

Commercial products like Nimeasy Natural Disinfectant feature neem and ajwain extracts as active ingredients, positioning themselves as safe for kitchen use due to food-grade ingredients [34]. This commercial precedent validates market acceptance of neem-based cleaning formulations and supports the commercial potential of our formulation.

3.5.5 Optimal Formulation Identification

Based on comprehensive evaluation across all parameters, Formulation C (5% neem + 5% lemon) emerges as the optimal balance point. Advantages of 5% formulation include free-flowing appearance maintained (unlike 7%), no staining on white tile (unlike 6-7%), balanced pleasant odor profile, good triterpenoid content (blue-green color test), excellent bioburden control (<10 CFU/ml), pH 3.5-3.6 in optimal range for antimicrobial enhancement, and smooth texture with no grittiness.

Trade-offs at higher concentrations (6-7%) introduce staining concerns without proportional benefits in other parameters. Trade-offs at lower concentrations (3-4%) provide excellent physical properties but lower triterpenoid content, though antimicrobial efficacy remains excellent at all concentrations tested.

4. Conclusion

This comprehensive investigation into neem and lemon-based herbal surface cleaner formulations has yielded several significant findings. Five concentrations (3-7% each of neem and lemon oils) were successfully prepared using a simple mixing protocol without complex equipment. Physical evaluation revealed that formulations up to 6% remain free flowing, while 7% exhibits slight viscosity increase. All formulations maintain creamy appearance and smooth texture with no grittiness. Odor transitions from fresh, citrus neem (3-4%) to balanced neem-lemon (5-7%) with increasing concentration.

All formulations exhibit acidic pH (3.5-3.8), favorable for antimicrobial enhancement and cleaning efficacy. Staining on white tiles appears at 6-7% concentrations (faded staining), while 3-5% formulations show no staining—identifying 5% as the maximum concentration for stain-free applications. Consistent foam formation (>2 cm, >5 min stability) across all concentrations confirms saponin content with excellent surfactant properties. Color testing confirmed triterpenoid presence with concentration-dependent intensification (pale green → dark blue green), validating neem's bioactive component incorporation. All formulations demonstrated total bacterial and fungal counts below 10 CFU/ml, indicating self-preserving systems with inherent antimicrobial activity meeting regulatory standards.

Formulation C (5% neem + 5% lemon) represents the optimal balance of physical properties (free flowing, no staining, balanced odor), chemical characteristics (good triterpenoid content, stable foam), and microbiological safety (<10 CFU/ml). This research establishes that neem and lemon-based herbal formulations offer promising potential as effective, safe, and environmentally sustainable alternatives to chemical-based surface cleaners.

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