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RESEARCH ARTICLE

Design, Development, and Pharmacological Evaluation of a Polyherbal Antimicrobial Hand Wash

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Abstract: Hand hygiene is one of the primary defense mechanisms against the transmission of infectious pathogens in clinical and community settings. While synthetic hand washes provide effective microbial reduction, their prolonged use is frequently associated with skin irritation, environmental toxicity, and the potential development of antimicrobial resistance. A polyherbal hand wash was developed using the bioactive extracts of *Psidium guajava* (Guava), *Piper betle* (Betel), *Murraya koenigii* (Curry leaves), *Ocimum sanctum* (Tulasi), and *Azadirachta indica* (Neem) to provide a safer, biodegradable, and skin-compatible alternative. These botanical agents were integrated into a gel-based system using *Sapindus mukorossi* (Reetha) as a natural surfactant and Aloe vera as a moisturizing emollient. Four distinct formulations (F1 to F4) were prepared and subjected to physicochemical and pharmacological assessments. Evaluation parameters included organoleptic properties, pH stability, foam height, foam stability, spreadability, and washability. Antimicrobial efficacy was determined using the cup-plate method against diverse microbial populations. Results indicated that all formulations maintained a skin-neutral pH (7.3 to 7.57). Skin safety was evaluated through acute irritancy test in Wistar rats, which showed no clinical signs of irritation, edema, or erythema. Formulation F4, which contains a combination of all herbal extracts, showed superior foaming capacity and enhanced antimicrobial activity. The absence of microbial growth in the finished products confirmed the efficacy of the preservative system and the inherent stability of the formulation. The developed polyherbal hand wash is an economically viable, eco-friendly, and effective substitute for synthetic antiseptic cleansers.

Keywords: *Psidium guajava*; Polyherbal formulation; Antimicrobial efficacy; Skin-neutral pH; Hand hygiene.

1. Introduction

The fundamental importance of handwashing in preventing disease transmission was first recognized in the mid-19th century. Early observations proved that hand disinfection could drastically reduce mortality rates in maternity wards and military hospitals [1]. In contemporary medicine, global health authorities have established hand hygiene is one of the primary measures of infection control, particularly in response to the rise of foodborne illnesses and global pandemics [2]. Standardized handwashing techniques are essential to mitigate the spread of transient flora and multidrug-resistant organisms [3].

Hand wash formulations typically function through the action of surfactants amphiphilic molecules containing both hydrophobic and hydrophilic regions. These molecules facilitate the emulsification of lipophilic debris and microorganisms, allowing them to be rinsed away [4]. However, excessive surfactant concentrations or aggressive synthetic solvents can lead to the "defatting" of the cutaneous lipid barrier. This loss of essential dermal oils results in xerosis and increased susceptibility to irritant contact dermatitis, especially among individuals requiring frequent hand disinfection [5]. Mainstream antiseptic hand washes often incorporate synthetic agents such as Triclosan or Chlorhexidine. While effective, these ingredients are associated with significant drawbacks. Triclosan has faced regulatory scrutiny due to its potential as an endocrine disruptor and environmental persistence [6]. The over-reliance on a limited range of synthetic biocides has raised concerns regarding the emergence of bacterial strains with reduced susceptibility. Consequently, there is a need for botanical alternatives that offer multi-target antimicrobial mechanisms while remaining gentle on the skin [7].

Secondary metabolites such as polyphenols, alkaloids, flavonoids, and terpenoids found in indigenous plants offer a broad spectrum of biological activities. A synergistic effect is often achieved by combining multiple extracts improving the overall antimicrobial potency while minimizing the required concentration of any single ingredient [8]. Natural foaming agents like saponins provide a biodegradable alternative to synthetic sulfates, reducing the chemical burden on the human integumentary system [9].

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2. Material and Methods

2.1. Collection and Authentication

Plant materials, including the leaves of *Psidium guajava, Piper betle, Murraya koenigii, Ocimum sanctum,* and *Azadirachta indica*, were sourced from AKRG College of Pharmacy premises. Fresh Aloe vera succulent leaves were collected for gel extraction. Each specimen was authenticated according to standard pharmacognostic procedures to ensure botanical identity and purity [10].

Table 1. Scientific Names and Pharmacological Roles of Ingredients

Ingredient	Scientific Name	Family	Role in the Formulation
Guava Leaves	*Psidium guajava*	Myrtaceae	Primary antibacterial agent; rich in tannins.
Betel Leaves	*Piper betle*	Piperaceae	Antiseptic and deodorizing properties.
Curry Leaves	*Murraya koenigii*	Rutaceae	Antioxidant and skin detoxifying agent.
Tulasi Leaves	*Ocimum sanctum*	Lamiaceae	Broad-spectrum antimicrobial and anti-inflammatory.
Neem Leaves	*Azadirachta indica*	Meliaceae	Antifungal and prevention of skin irritations.
Aloe Vera	*Aloe barbadensis*	Asphodelaceae	Humectant, emollient, and skin healing base.
Reetha	*Sapindus mukorossi*	Sapindaceae	Natural surfactant; source of saponins for foaming.

2.2. Extraction Method

The preparation of herbal extracts followed a systematic procedure to maximize the yield of bioactive markers [11]. Leaves were washed with distilled water to remove epiphytic flora. Grinding and pulping were performed to increase the surface area for solvent penetration. Aqueous extraction was conducted via the decoction method. The resulting mixtures were filtered through fine muslin cloth and Grade I filter paper, followed by controlled evaporation to obtain enriched herbal concentrates [12].

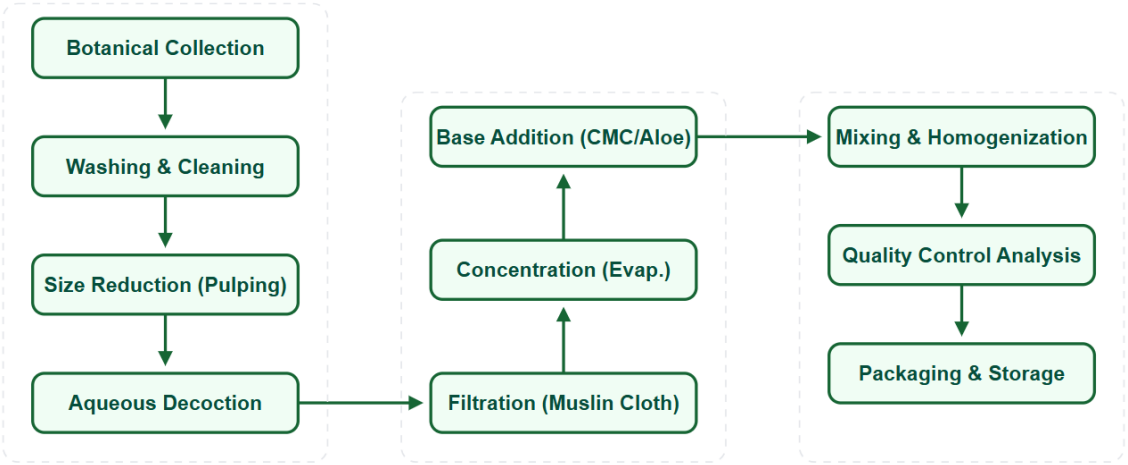

Figure 1. Process Flowchart for Herbal Hand Wash Preparation

2.3. Formulation Design

Four distinct batches (F1 to F4) were developed to evaluate the influence of varying herbal concentrations [13]. Formulation F4 consisted of a blend of all primary extracts to determine synergistic potential. Carboxy Methyl Cellulose (CMC) was employed as a rheology modifier to achieve the desired gel consistency.

2.4. Physicochemical Evaluation

2.4.1. Organoleptic and Physical Appearance

The formulations were visually inspected for homogeneity, color, clarity, and consistency. No visible particulate matter or phase separation was permitted during inspection [14].

Table 2. Composition of Polyherbal Hand Wash Formulations F1-F4

Ingredients (per 100 mL)	F1	F2	F3	F4 (Optimized)
Guava leaf extract	5.0 mL	-	-	2.5 mL
Betel leaf extract	-	5.0 mL	-	2.5 mL
Curry leaf extract	-	-	5.0 mL	2.5 mL
Tulasi leaf extract	2.5 mL	2.5 mL	2.5 mL	2.5 mL
Neem leaf extract	1.5 mL	1.5 mL	1.5 mL	1.5 mL
Aloe vera gel	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Sapindus mukorossi	5.0 g	5.0 g	5.0 g	5.0 g
Glycerin	1.5 mL	1.5 mL	1.5 mL	1.5 mL
Sodium Benzoate	0.1 mL	0.1 mL	0.1 mL	0.1 mL
CMC (Thickener)	5.0 g	5.0 g	5.0 g	5.0 g
Distilled Water	q.s.	q.s.	q.s.	q.s.

2.4.2. pH Determination

A 1% (w/v) solution of the herbal hand wash was prepared in distilled water. The pH was measured using a standardized digital pH meter at 25 ± 2ºC in triplicate. The target range for skin compatibility was established between 5.5 and 7.5 [15].

2.4.3. Foaming Capacity and Stability

One gram of the formulation was dispersed in 50 ml of distilled water in a 500 ml graduated cylinder. The volume was adjusted to 100 ml. After 25 standardized strokes, the initial foam height was recorded. Stability was evaluated by measuring the foam height after 30 minutes, and the stability percentage was calculated [16].

2.4.4. Washability and Spreadability

Lathering efficiency and the presence of any sticky residue were assessed by applying five milliliters of the product to a substrate. Spreadability was assessed qualitatively by placing 1 g of the gel between two glass slides and observing the ease of displacement under uniform pressure [17].

2.5. Pharmacological Evaluation

2.5.1. Primary Skin Irritation Test

The acute dermal irritancy of the developed polyherbal formulations was evaluated using Wistar rats (weighing 150–200g). The study protocol was approved by the Institutional Animal Ethics Committee (IAEC Approval No: AKRG/IAEC/2025/053) and conducted in accordance with CPCSEA guidelines. Animals were housed under standard environmental conditions with free access to food and water. Approximately 24 hours prior to the study, hair was removed from the dorsal thoracic region using a sterile clipper. The animals were divided into four groups corresponding to formulations F1-F4. A quantity of 0.5 g of each formulation was applied to the shorn area and secured with a non-occlusive dressing. The application site was monitored at intervals of 1, 24, 48, and 72 hours for any clinical signs of irritation, erythema, or edema, following the general principles of OECD guidelines 404 for dermal toxicity [18].

2.5.2. Antimicrobial Efficacy

The cup-plate method was used to determine inhibitory potential. Nutrient Agar was sterilized at 121º C for 15 minutes. A soil suspension was used as the inoculum to represent a diverse microbial challenge. Uniform cups were punched into the agar using a sterile borer, and formulations F1-F4 were added. Plates were incubated at 37±1ºC for 24 hours, and the resulting Zones of Inhibition (ZOI) were measured [19].

3. Results and Discussion

3.1. Organoleptic and Physical Characterization

The polyherbal formulations (F1–F4) were subjected to qualitative organoleptic evaluation. All batches exhibited a homogeneous gel-like consistency with a distinct greenish-brown color, attributed to the concentrated chlorophyll and tannin content of the herbal

extracts. The preparations maintained a pleasant botanical odor without the need for synthetic fragrance enhancers. The textures were smooth and non-sticky upon application, leaving a refreshed and moisturized sensation on the dermal surface.

Table 3. Organoleptic Characteristics of Formulations F1-F4

Parameter	F1	F2	F3	F4
Appearance	Gel	Gel	Gel	Gel
Color	Greenish-brown	Greenish-brown	Greenish-brown	Greenish-brown
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Texture	Smooth	Smooth	Smooth	Smooth
After-feel	Fresh/Moisturized	Fresh/Moisturized	Fresh/Moisturized	Fresh/Moisturized
Homogeneity	Uniform	Uniform	Uniform	Uniform

3.2. Physicochemical Analysis

The physicochemical parameters, including pH, foaming capacity, and foam stability, were recorded in triplicate and are expressed as Mean ± Standard Deviation (SD).

3.3. pH Stability

The pH of the formulations ranged from 7.30 ± 0.11 to 7.57 ± 0.05. Batch F2 exhibited the most acidic-leaning profile (7.30 ± 0.11), while F4 showed a slightly higher value (7.57 ± 0.05). These values are within the optimal range for topical applications, ensuring minimal disruption to the skin's acid mantle [20].

3.4. Foaming Capacity and Stability

Foaming ability was highest in Formulation F4 (9.00 ± 0.04 cm), suggesting a positive synergistic interaction between the multiple herbal extracts and the *Sapindus mukorossi* saponins. Foam stability was calculated after 30 minutes, with F1 showing the highest persistence at 97.14%, whereas F4, despite higher initial volume, showed a stability of 88.88%.

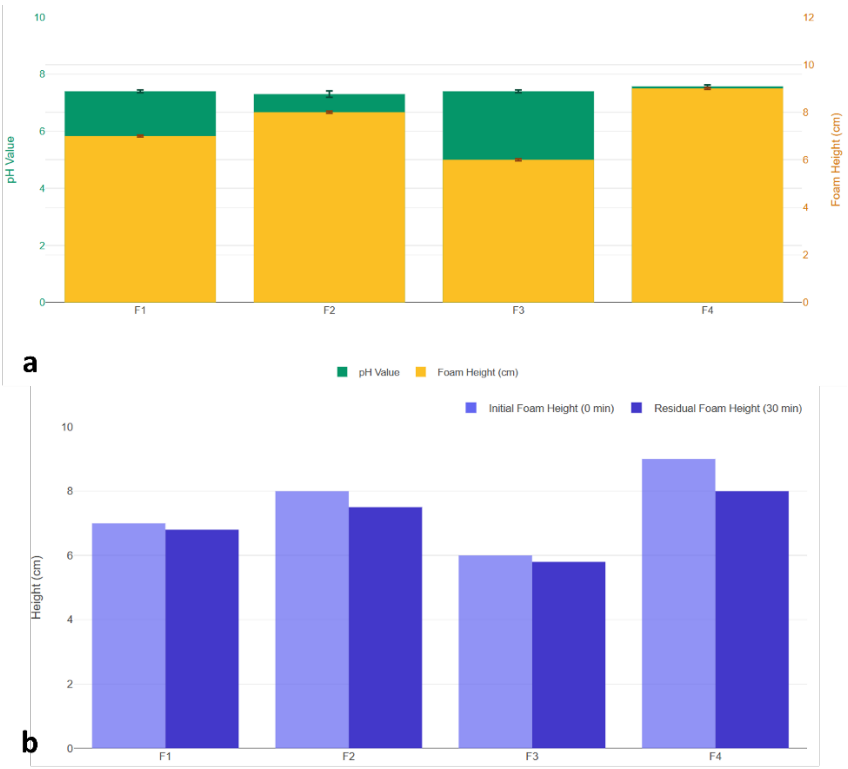

Figure 2. Physicochemical Properties of Formulations F1-F4

Table 4. Physicochemical Parameters (pH, Foam Height, and Stability)

Batch	pH Value*	Foam Height* (cm)	Foam Stability* (%)
F1	7.40 ± 0.05	7.00 ± 0.04	97.14%
F2	7.30 ± 0.11	8.00 ± 0.04	93.14%
F3	7.40 ± 0.05	6.00 ± 0.04	96.66%
F4	7.57 ± 0.05	9.00 ± 0.04	88.88%

**Mean ± SD, n = 3 observations*

3.5. Spreadability and Washability

Quantitative spreadability observations confirmed that the gel system made up of CMC provided an even distribution across the dermal substrate. Washability tests indicated that the formulations were easily removed with water, leaving zero sticky residues. The moisturizing after-feel was consistent across all batches, primarily due to the presence of Aloe vera and Glycerin.

3.6. Skin Irritation Test

In the acute dermal irritancy study conducted on Wistar rats, none of the formulations (F1–F4) produced clinical signs of irritation. Observations at 1, 24, 48, and 72 hours revealed a Primary Irritation Index (PII) of 0.00 for all groups. No edema, erythema, or inflammation was noted, confirming the non-sensitizing nature of the polyherbal system [21].

3.7. Antimicrobial Activity

The cup-plate method against soil-derived microbial populations showed significant zones of inhibition (ZOI) for all formulations. Formulation F4 showed a comparatively larger ZOI, likely due to the combined presence of *Psidium guajava*, *Piper betle*, and *Murraya koenigii* extracts. The absence of secondary microbial growth within the formulations during the testing period further validates the efficacy of the preservative system and the inherent antiseptic properties of the herbs.

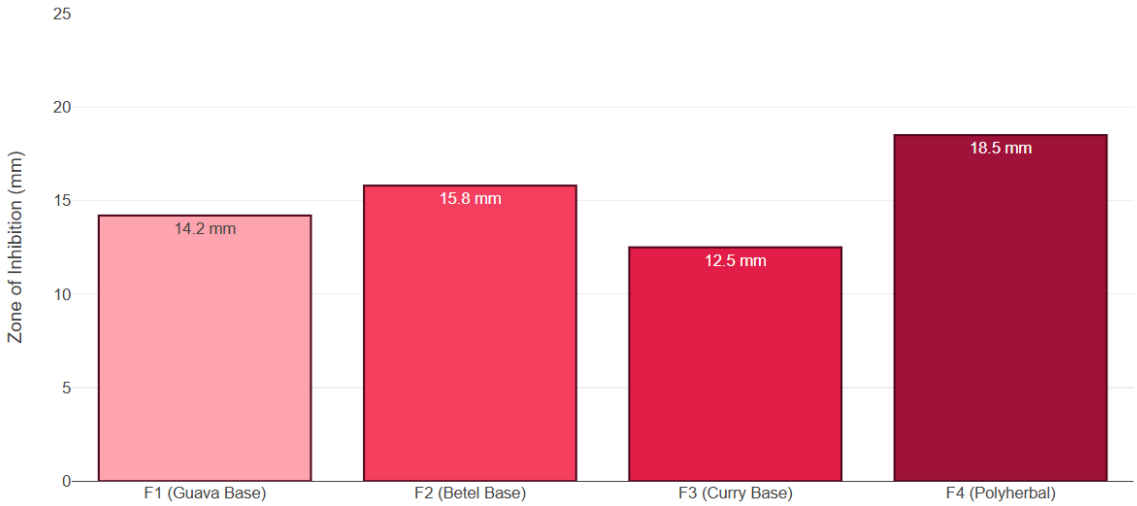

Figure 3. Antimicrobial Activity of Herbal Handwash Formulations (F1 to F4)

3.8. Stability studies

Stability assessments were conducted over a 45-day period at room temperature. The formulations remained physically stable with no significant changes in color, odor, or texture. The pH values showed minor fluctuations (7.3 ± 0.03 to 7.2 ± 0.03 by Day 45), which remained well within the acceptable pharmaceutical limits. Foam height and stability remained consistent, indicating that the CMC-based gel network effectively prevented the degradation of the active saponins and extracts.

Table 5. 45-Day Stability Test Results of Optimized Formulation F4

Parameter	Day 0 (Initial)	Day 15	Day 30	Day 45
Color	Greenish-brown	No change	No change	No change
Odour	Pleasant	No change	No change	No change
pH	7.57 ± 0.05	7.4 ± 0.03	7.3 ± 0.03	7.2 ± 0.03
Foam Height (cm)	9.00 ± 0.04	9.00 ± 0.02	8.9 ± 0.04	8.8 ± 0.05
Dermal Irritancy	Not Observed	Not Observed	Not Observed	Not Observed
Phase Separation	None	None	None	None

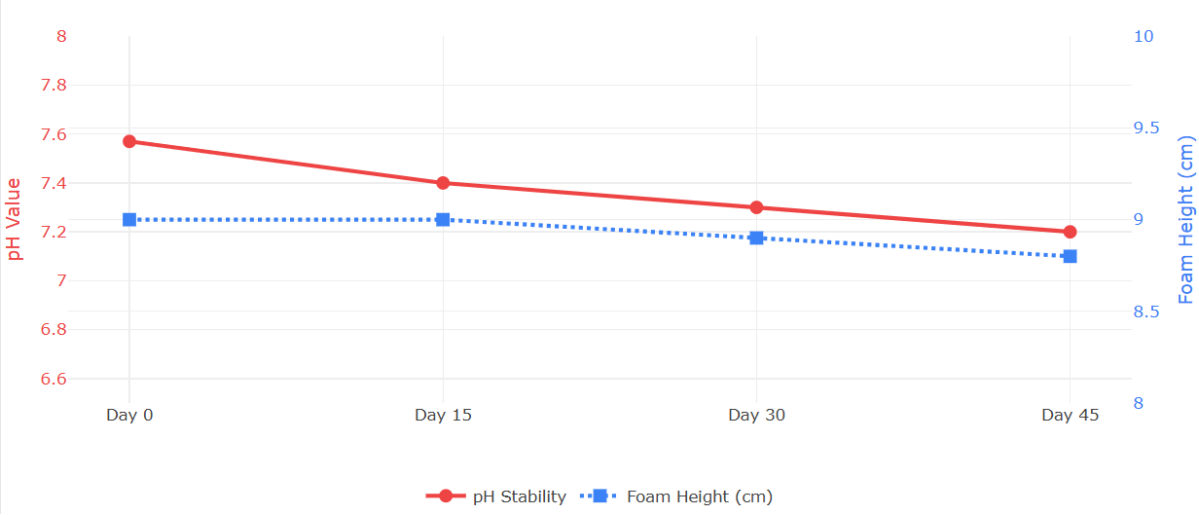

Figure 4. Stability Test Results of pH and Foam Height of Optimized Formulation F4

4. Discussion

The primary challenge in developing herbal hand washes is achieving a balance between cleansing efficiency and skin compatibility. Formulation F4 emerged as the optimized batch, as it leveraged the diverse phytochemical profiles of five distinct plants. The high foaming capacity observed in F4 (9 cm) is particularly noteworthy, as it suggests that the saponins from *Sapindus mukorossi* function effectively in the presence of various botanical polyphenols [22]. Unlike synthetic Sodium Lauryl Sulfate (SLS), which often causes skin barrier damage, these natural surfactants provide effective micellar solubilization of grime without excessive lipid stripping.

The enhanced antimicrobial activity of the polyherbal blend can be attributed to the multi-target mechanisms of its constituents. The tannins in *Psidium guajava* are known to precipitate bacterial proteins, while the eugenol in *Piper betle* disrupts microbial cell membranes [23]. The azadirachtin from *Azadirachta indica* serves as a potent inhibitor of bacterial growth enzymes. This synergistic "multi-herb" approach reduces the likelihood of microbial resistance compared to single-agent synthetic antiseptics [24].

A critical finding of this study was the skin-neutral pH maintained by the formulations. The human skin surface typically has a pH of 4.7 to 5.75; maintaining a hand wash pH near 7.0 minimizes the risk of alkalosis-induced barrier dysfunction [25]. The results of the Wistar rat irritancy study (PII = 0.00) align with the historical safe use of these plants in traditional Ayurvedic medicine, suggesting that the developed product is suitable for frequent daily use.

5. Conclusion

A polyherbal hand wash system was successfully designed, developed, and evaluated using indigenous botanical extracts. The integration of *Psidium guajava*, *Piper betle*, and other medicinal leaves into a gel-based vehicle resulted in a product that combines effective antimicrobial action with superior dermal safety. The use of natural surfactants like Reetha ensures that the formulation is biodegradable and environmentally sustainable. Among the tested batches, Formulation F4 exhibited the most favorable profile in terms of foaming capacity and antimicrobial synergy. The animal irritancy studies confirmed the non-toxic nature of the formulation, making it a viable and safe alternative to commercial synthetic hand washes. Future studies may focus on the isolation of specific bioactive markers to further standardize the formulation for large-scale pharmaceutical production.

Compliance with ethical standards

Acknowledgements

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Conflict of interest statement

The authors declare that they have no potential conflicts of interest or competing interests with respect to the research, authorship, and/or publication of this manuscript. No financial support or products from any third-party institutions influenced the outcome of this study.

Statement of ethical approval

The experimental protocol was reviewed and approved (AKRG/IAEC/2025/053) by the Institutional Animal Ethics Committee (IAEC) of the institute and was conducted in strict accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), formerly known as CPCSEA, Government of India. All procedures were performed under appropriate ethical oversight to ensure animal welfare.

Statement of informed consent

The present research work does not contain any studies performed on human subjects by any of the authors. Therefore, the statement of informed consent is not applicable to this study

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