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Research Article

Anti-Acne Efficacy of a Topical Cream Formulated with Sapodilla (*Manilkara zapota* (L.) P. Royen) Leaf Extract

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Acne vulgaris is a chronic inflammatory skin disorder affecting up to 85% of the adolescent and adult population globally. Its pathophysiology involves a complex interplay of excessive sebum production, follicular hyperkeratinization, and colonization by *Cutibacterium acnes* and *Staphylococcus aureus*. Growing antimicrobial resistance and adverse effects associated with conventional topical agents have spurred interest in plant-derived therapeutic alternatives. This study aimed to develop and evaluate the physicochemical properties, storage stability, and antibacterial activity of oil-in-water (O/W) topical creams incorporating sapodilla (*Manilkara zapota* (L.) P. Royen) leaf extract. Three cream formulations were prepared using a stearic acid–triethanolamine (TEA) emulsification system at extract concentrations of 12.5%, 25%, and 50% (w/w). Physicochemical evaluation encompassed organoleptic assessment, homogeneity, emulsion type, pH, spreadability, and viscosity. Accelerated stability testing was performed over six thermal cycling runs (4°C/40°C) and centrifugation at 3,000 rpm for 30 minutes at weeks one and four. Antibacterial activity was assessed by the agar well-diffusion method against *P. acnes* and *S. aureus*. All formulations met physicochemical specifications and passed stability testing across four weeks. Inhibition zones increased proportionally with extract concentration; the 50% formulation produced strong inhibition against both pathogens (10.75–12.0 mm against *S. aureus*; 11.0–13.0 mm against *P. acnes*), comparable to gentamicin cream ($p > 0.05$). The 50% sapodilla leaf extract produced a stable O/W cream with antibacterial efficacy equivalent to gentamicin, reinforcing its potential as a candidate phytopharmaceutical anti-acne agent.

Acne vulgaris is a chronic dermatological condition characterized by a prolonged clinical course and is consistently ranked among the most prevalent skin disorders worldwide [1]. Its pathogenesis is multifactorial and driven by four interacting mechanisms: increased sebum production, follicular hyper keratinization within the pilosebaceous unit, overgrowth of *Cutibacterium acnes* (formerly designated *Propionibacterium acnes*), and dysregulated immune responses within the hair follicle [2]. The concerted action of these mechanisms fosters a microenvironment conducive to diverse lesion formation, ranging from non-inflammatory comedones to painful inflammatory nodules.

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Three Gram-positive bacterial species play pivotal roles in follicular colonization and acne progression: *Cutibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Among these, *C. acnes* is the most dominant pathogen, activating the innate immune response and stimulating pro-inflammatory cytokine release—including TNF-α—and reactive oxygen species through the inducible nitric oxide synthase (iNOS) pathway. The two *Staphylococcus* species augment this process by forming biofilms that create anaerobic niches favoring *C. acnes* proliferation, thereby amplifying the overall inflammatory cascade [3].

Current acne management relies on topical and systemic therapies. Topical agents are generally preferred as first-line therapy for mild-to-moderate acne owing to their favorable safety profiles, while systemic regimen are reserved for more severe or refractory cases [4]. However, conventional topical agents—including benzoyl peroxide, retinoids, and antibiotic preparations—frequently cause adverse effects such as erythema, pruritus, and skin irritation, ultimately compromising patient adherence and therapeutic outcomes [5]. Simultaneously, the escalating burden of antimicrobial resistance has made the exploration of plant-based therapeutic alternatives increasingly urgent.

Medicinal plants represent a rich source of bioactive phytochemical compounds with multi-target mechanisms of action, encompassing antibacterial, anti-inflammatory, anti-lipogenic, and anti-androgenic activities that are directly relevant to acne management [6]. The sapodilla tree (*Manilkara zapota* (L.) P. Royen) has been documented to contain a diverse array of phytoconstituents, including flavonoids, phenolic acids, alkaloids, terpenoids, and anthraquinone glycosides, which collectively confer potent antimicrobial properties [7]. Recent scientific evidence demonstrates that sapodilla leaf extract displays synergistic adjuvant activity against clinically relevant pathogens, including *S. aureus*, supporting its potential as an anti-acne agent [7].

Topical creams represent one of the most clinically practical and cosmetically acceptable drug delivery systems for applying bioactive compounds to the skin. As semisolid emulsion preparations contain more than 20% water with less than 50% hydrocarbon or wax content, creams facilitate efficient distribution of active ingredients across the skin surface [8]. Oil-in-water (O/W) emulsion systems, in particular, promote drug release through an evaporative concentration mechanism following application, enhancing the penetration of water-soluble active compounds into skin layers [9].

Despite the promising phytochemical profile of sapodilla leaves, scientific evidence regarding their formulation into stable and effective anti-acne cream preparations remains limited. Accordingly, this study was designed to evaluate the physical characteristics, stability, and antibacterial activity of anti-acne topical creams formulated with *Manilkara zapota* (L.) P. Royen leaf extract at varying concentrations against *P. acnes* and *S. aureus*, the two principal bacteria implicated in the pathogenesis of acne vulgaris.

Results and Discussion

Extraction

Maceration of sapodilla (*Manilkara zapota* (L.) P. Royen) leaves yielded a dark brownish thick extract. Extraction of 500 g of dried herbal powder produced 130 g of thick extract, corresponding to a yield of 21.66%. This relatively high yield is consistent with existing phytochemical literature documenting the abundance of polar compounds in sapodilla leaves, including flavonoids, phenolics, and terpenoid which dissolve efficiently in 96% ethanol [7].

Organoleptic Properties

Organoleptic observations were conducted before and after stability testing to assess the consistency of the physical appearance of the cream formulations throughout the storage period. Results are presented in **Table 1**. All cream formulations maintained consistent organoleptic characteristics throughout the observation period, with no detectable changes in color, odor, or texture before and after stability testing. Color intensity was directly

proportional to extract concentration. Formulas A and B (12.5% and 25%) appeared light brown, while Formula C (50%) displayed a darker brown hue attributable to its higher phytochemical content, particularly tannins and phenolic pigments. The blank formulation remained white and odorless, as expected of a pure cream base. Preservation of these characteristics across all formulas constitutes a positive indicator of physical stability and ensures adequate cosmetic acceptability for topical application [10].

Table 1. Organoleptic evaluation of sapodilla leaf extract cream formulations

Testing Period	Formula	Color	Odor/Aroma	Texture
Before Stability Test	A (12.5%)	Light brown	Characteristics of sapodilla leaf	Semisolid
	B (25%)	Light brown	Characteristics of sapodilla leaf	Semisolid
	C (50%)	Dark brown	Characteristics of sapodilla leaf	Semisolid
	Blank (Control)	White	Odorless	Semisolid
After Stability Test	A (12.5%)	Light brown	Characteristics of sapodilla leaf	Semisolid
	B (25%)	Light brown	Characteristics of sapodilla leaf	Semisolid
	C (50%)	Dark brown	Characteristics of sapodilla leaf	Semisolid
	Blank (Control)	White	Odorless	Semisolid

pH

The pH of all cream formulations was measured at Week 1 and Week 4 using universal pH indicator paper. A marginal decline from pH 7 at Week 1 to pH 6 at Week 4 was observed across all formulations; however, all remained within the physiologically acceptable range for topical preparations (4.5–7.0). This range aligns with normal skin surface pH (approximately 4.5–6.5), thereby minimizing the risk of irritation or disruption to the skin barrier function. The observed time-dependent pH reduction may be attributed to several factors: decomposition of unstable components within the oil phase, particularly unsaturated fatty acids derived from stearic acid; chemical interactions between the active ingredients and excipients during storage; and the influence of dissolved environmental CO_2 reacting with the aqueous phase to form carbonic acid [11]. The uniform pattern of pH changes across all formulations, including the extract-free blank, indicates that these changes are inherent characteristics of the cream base composition rather than consequences of the chemical nature of the extract.

Homogeneity

Microscopic examination of cream samples spread onto glass slides revealed uniform distribution of components with no visible coarse particles or phase discontinuities. All formulations demonstrated consistent homogeneity throughout the four-week storage period, with no observable phase separation or particle aggregation. These findings affirm the efficacy of the trituration-based preparation method in ensuring uniform dispersion of sapodilla leaf extract within the cream matrix. Homogeneity is a critical quality attribute for topical preparations because it directly governs the uniformity of drug dose delivered at each application site. Homogeneous cream ensures that each unit of application contains an equivalent quantity of active substances, thereby maintaining consistent therapeutic efficacy across treated skin areas [12].

Emulsion Type

All cream formulations, both extract-containing and blank, exhibited an oil-in-water (O/W) emulsion type, as confirmed by their ability to mix completely with distilled water. This characteristic arises from the emulsification system employed, in which stearic acid and TEA react *in situ* to form a soap that functions as an effective O/W emulsifier,

stabilizing oil droplets within a continuous aqueous phase. The O/W emulsion type confers distinct therapeutic advantages in anti-acne applications: upon topical application, continuous evaporation of the aqueous phase concentrates on active substances at the skin surface, promoting enhanced penetration into follicular structures colonized by *P. acnes* and *S. aureus* [9]. The retention of O/W characteristics throughout four weeks of storage confirms that incorporation of sapodilla leaf extract does not disturb the emulsification system of the cream base.

Spread ability

Spread ability of each formulation was assessed before and after stability testing. The accepted spread ability range for semisolid cream preparations is 5–7 cm for semifluid systems. Results are presented in Table 2.

Table 2. Spreadability of sapodilla leaf extract cream formulations during storage

Formula	Week 1 (Before Stability Test)	Week 4 (After Stability Test)
A (12,5%)	5,0 cm	5,2 cm
B (25%)	5,0 cm	5,1 cm
C (50%)	4,5 cm	4,0 cm
Control (Blank)	5,2 cm	5,8 cm

Formulas A, B, and the blank control met the semifluid spread ability criteria (5–7 cm) at both time points, whereas Formula C (50%) fell within the semi consistent range (3–5 cm), attributable to its higher extract content increasing emulsion viscosity [13]. The incremental increase in spread ability observed in Formulas A, B, and the blank following storage is consistent with the inverse relationship between viscosity and spread ability. The decrease in Formula C's spread ability may relate to the higher concentration of extract-derived compounds interacting with the cream matrix, reinforcing its semisolid network. Adequate spread ability is functionally important as it determines the ease of application and the surface area of skin accessible to the active ingredient, thereby directly influencing overall therapeutic effectiveness.

Viscosity

Viscosity measurements were conducted before and after stability testing. All formulations were required to meet a minimum viscosity threshold of >50 dPa·s. Results are presented in Table 3.

Table 3. Viscosity of sapodilla leaf extract cream formulations during storage

Formula	Week 1 (Before Stability Test)	Week 4 (After Stability Test)
A (12,5%)	190 dPa·s	203 dPa·s
B (25%)	110 dPa·s	253 dPa·s
C (50%)	140 dPa·s	186 dPa·s
Control (Blank)	180 dPa·s	230 dPa·s

All formulations substantially exceeded the minimum viscosity threshold of 50 dPa·s throughout the study, indicating satisfactory consistency for semisolid topical applications. A general trend of viscosity increase from Week 1 to Week 4 was observed across all formulations. This phenomenon is primarily attributable to thermal cycling conditions during stability testing: exposure to cold temperatures (4°C) during cycles induces partial crystallization within the oil phase, increasing resistance to flow [14]. Furthermore, the mechanical shear forces applied during centrifugation likely contribute to emulsion microstructural reorganization, ultimately elevating apparent viscosity. Higher viscosity values provide notable practical benefits, including improved cream adhesion to the skin surface, reduced risk of dripping during application, and extended contact time for active ingredient release.

Physical Stability

Cycling Test

The cycling test subjected cream formulations to six accelerated thermal stress cycles

(4°C↔4°C), simulating the realistic temperature stresses encountered during distribution and storage. All cream formulation demonstrated excellent stability across all six thermal stress cycles, with no evidence of phase separation, syneresis, or textural deterioration. These results reflect the robust emulsification provided by the stearic acid–TEA soap system, which maintains sufficient interfacial film strength to preserve O/W droplet integrity even under thermal stress. The fact that all formulations, including Formula C at the highest extract concentration passed this stringent test indicates that incorporation of sapodilla leaf extract, even at 50%, does not compromise the structural integrity of the emulsion system [15].

Centrifugation Test

The centrifugation test evaluated the effect of mechanical stress on emulsion stability, accelerating approximately one year of gravitational exposure. No phase separation was observed in any cream formulation following centrifugation at 3,000 rpm for 30 minutes. The absence of creaming, sedimentation, or coalescence confirms that the emulsification system maintains sufficient kinetic stability to resist gravitational forces. These results suggest that the sapodilla leaf extract cream formulations will likely remain physically stable for at least one year under appropriate storage conditions [16]. The consistent stability of all formulations in both cycling and centrifugation tests provides robust evidence that the O/W cream matrix is thermodynamically and mechanically compatible with sapodilla leaf extract at all tested concentrations.

Antibacterial Activity

The antibacterial activity of all cream formulations against *Staphylococcus aureus* and *Propionibacterium acnes* was evaluated by the agar well-diffusion method (Figure 1).

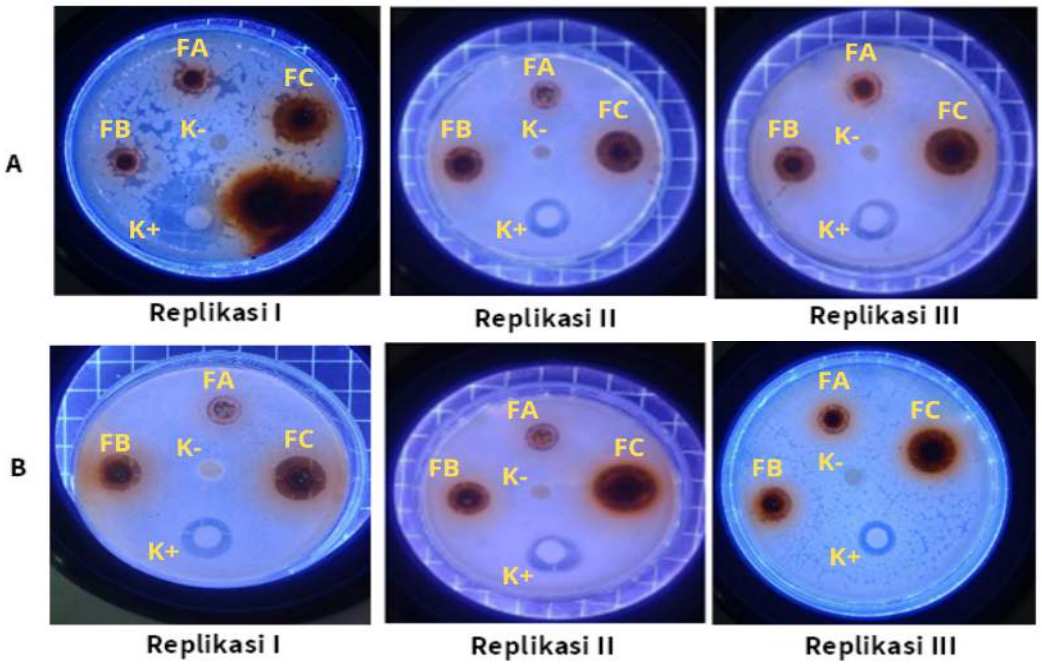

Figure 1. Results of antibacterial activity testing. A. *Staphylococcus aureus* bacteria. B. *Propionibacterium acnes* bacteria. FA: Cream with 12.5% extract concentration; FB: Cream with 25% extract concentration; FC: Cream with 50% extract concentration; K−: Negative control; K+: Positive control.

Antibacterial evaluation revealed a clear concentration-dependent inhibition pattern (Table 4). For *S. aureus*, the 12.5% formulation produced weak-to-moderate inhibition zones (5.0–6.0 mm), the 25%

formulation showed moderate inhibition (6.75–9.0 mm), and the 50% formulation generated strong inhibition zones (10.75–12.0 mm). A similar concentration-response trend was observed for *P. acnes*, with 12.5% yielding weak-to-moderate inhibition (5.0–7.75 mm), 25% demonstrating moderate activity (8.0–8.75 mm), and 50% exhibiting strong inhibitory effects (11.0–13.0 mm). The negative control produced no inhibition zones under any condition, confirming that the cream base itself lacks intrinsic antibacterial activity. At 50% concentration, inhibition zones against *P. acnes* (up to 13.0 mm) were comparable to or exceeded those of the positive control (gentamicin cream; 7.5–9.5 mm), indicating that the high-concentration sapodilla leaf extract cream is potentially clinically relevant as an anti-acne agent [17].

The antibacterial activity of sapodilla leaf extract is primarily attributable to the diversity of its phytoconstituents. Flavonoids and phenolic acids disrupt bacterial membrane integrity and inhibit essential enzymatic processes; terpenoids act through membrane-active mechanisms; and alkaloids interfere with bacterial nucleic acid synthesis [7]. The multi-target nature of these phytochemicals collectively impedes the development of bacterial resistance, a significant advantage over conventional single-target antibiotics. Moreover, the activity of the cream formulation against both *P. acnes* and *S. aureus*, organisms with distinct cell-wall characteristics further underscores the broad-spectrum antibacterial potential of this extract. These findings are consistent with published literature reporting antibacterial activity of *M. zapota* leaf extracts against Gram-positive pathogens [7] and support further development of this material as a phytopharmaceutical active anti-acne ingredient.

Taking holistically, the physicochemical, stability, and antibacterial findings of this study establish that sapodilla leaf extract (*Manilkara zapota* (L.) P. Royen) can be successfully incorporated into an O/W topical cream with acceptable physical properties, excellent storage stability, and meaningful antibacterial activity against the principal bacteria involved in acne pathogenesis. This work provides a scientific foundation for further preclinical and clinical development of sapodilla leaf extract cream as a phytopharmaceutical anti-acne product. Future investigations are recommended to assess the irritation potential of these formulations through standardized dermal sensitization testing, and to explore more optimized drug delivery systems—such as liposomal or nanoparticulate formulations—to further improve follicular penetration and therapeutic efficacy.

Table 4. Inhibition zone diameters of sapodilla leaf ethanol extract cream against *Staphylococcus aureus* and *Propionibacterium acnes*

Treatment Group	Inhibition Zone Diameters (Mean±SD, mm)			
	S. aureus	**Kategori**	***P. acnes***	**Kategori**
Formula A (12,5%)	5,33 ± 0,49	Moderate	6,25 ± 1,22	Moderate
Formula B (25%)	8,17 ± 1,04	Moderate	8,50 ± 0,50	Moderate
Formula C (50%)	11,33 ± 0,74	Strong	11,75 ±0,93	Strong
Positive Control (Gentamicin)	10,50 ± 2,19	Strong	8,33 ± 0,88	Moderate
Negative Control (Blank)	0,00 ± 0,00	-	0,00 ± 00	-

Conclusion

This study has demonstrated that sapodilla leaf extract (*Manilkara zapota* (L.) P. Royen) can be successfully formulated into anti-acne topical creams at concentrations of 12.5%, 25%, and 50%. All three formulations satisfied the required physical evaluation parameters—encompassing organoleptic properties, homogeneity, emulsion type, pH, spread ability, and viscosity—and maintained satisfactory physical stability over four weeks as assessed by

cycling and centrifugation tests. The cream preparations exhibited concentration-dependent antibacterial activity against both *Propionibacterium acnes* and *Staphylococcus aureus*; the 50% formulation produced strong inhibitory activity statistically equivalent to gentamicin cream (positive control). These findings substantiate the phytopharmaceutical potential of sapodilla leaf extract as a natural active anti-acne ingredient. Further research is recommended to conduct irritation testing, assess dermal safety, and explore advanced drug delivery strategies to optimize the clinical applicability of this formulation

Materials and Methods

Equipment

The equipment used in this study included: maceration vessels, magnetic stir bar stirrer, porcelain evaporating dishes, glass Petri dishes, separatory funnel, hot plate (Stuart®, UK), graduated glass cylinders (Pyrex®), glass beakers (Pyrex®), oven (Memmert®, Germany), rotary vacuum evaporator (Büchi®, Switzerland), Rion VT-06 rotational viscometer, centrifuge, digital vernier caliper, and digital analytical balance (Ohaus®, USA).

Materials

The chemicals employed comprised 96% ethanol (extraction solvent), distilled water, stearic acid 15%, triethanolamine (TEA) 2%, cetyl alcohol 4%, glycerin 10%, methylparaben 0.18%, propylparaben 0.02%, Nutrient Agar (HiMedia®), and 0.9% NaCl solution. The plant material consisted of fresh sapodilla leaves (*Manilkara zapota* (L.) P. Royen) harvested from Watu-Watu Village, West Kendari District, Kendari City, Indonesia.

Plant Material Processing and Extraction

Freshly harvested sapodilla leaves from Watu-Watu Village, West Kendari District, Kendari City, were subjected to wet sorting and washed thoroughly to remove impurities. The leaves were then sliced, dried at room temperature in a shaded area protected from direct sunlight, and dry-sorted to obtain ready-to-process herbal material. Extraction was performed by maceration using 96% ethanol as solvent. Dried herbal material was immersed in 96% ethanol for 3×24 hours with solvent replacement every 24 hours under dark conditions to prevent photodegradation of active compounds. The collected macerates were combined and re-macerated three times. The resulting extract was concentrated using a rotary vacuum evaporator at 40°C until a thick extract suitable for cream formulation was obtained.

Cream Formulation and Preparation

The cream preparation process followed a previously validated method [18]. Three cream formulations (A, B, C) were prepared at sapodilla leaf extract concentrations of 12.5%, 25%, and 50%, respectively, together with one blank (control) formulation without extract addition. The oil phase, consisting of stearic acid, cetyl alcohol, and propylparaben, was melted in a porcelain dish at 70°C (Mixture I). Concurrently, the aqueous phase containing TEA, glycerin, methylparaben, and distilled water was heated separately to the same temperature (Mixture II). Both phases were then combined in a mortar and triturated until a homogeneous cream base was formed. Sapodilla leaf extract was subsequently incorporated in a stepwise manner with continuous trituration until uniform dispersion was achieved. The finished cream preparations were transferred into sterile, labeled containers.

Physical Evaluation of Cream Preparations

Physical quality evaluation was conducted at Week 1 (before stability testing) and Week 4 (after stability testing). Testing encompassed: (1) Organoleptic assessment of color, odor, and texture by visual and sensory examination [19]; (2) pH measurement using universal pH indicator paper immersed in cream samples; (3) Viscosity determination using a Rion VT-06 rotational viscometer at room temperature (28°C); (4) Homogeneity evaluation by microscopic examination of a thin cream layer spread between two glass slides [19]; (5) Spread ability testing by placing 0.5 g of

cream at the center of a 1-cm diameter glass slide, overlaying a second glass slide, and applying incremental weights of 100 g and 200 g for two minutes at 25°C, with spread diameter measured thereafter; and (6) Emulsion type determination by diluting the cream with distilled water and observing miscibility behavior—O/W emulsions mix completely with water, while W/O emulsions resist dilution [19].

Stability Testing

Physical stability was evaluated using two complementary methods. First, cycling testing was performed by exposing each cream formulation to six consecutive temperature cycles; each cycle comprised storage at 4°C ± 2°C for 24 hours followed by 40°C ± 2°C for a further 24 hours, with organoleptic observations, pH, and viscosity recorded after each cycle [15]. Second, centrifugation testing was performed to assess mechanical phase stability by centrifuging each formulation at 3,000 rpm for 30 minutes, followed by observation for phase separation [16].

Antibacterial Activity Testing

The antibacterial activity of each cream formulation was tested against *Staphylococcus aureus* and *Propionibacterium acnes* using the agar well-diffusion method. Bacterial isolates were subculture on Nutrient Agar (NA) medium and standardized to 0.5 McFarland turbidity prior to inoculation. Sterile Petri dishes containing inoculated NA medium were prepared for each test organism, after which wells were filled with cream formulations at concentrations of 12.5%, 25%, and 50%. Gentamicin cream served as the positive control, while the blank cream formulation served as the negative control. All plates were incubated at 37°C for 24 hours. Each treatment was performed in quadruplicate. Inhibition zone diameters were measured and categorized as weak (<5 mm), moderate (5–10 mm), or strong (>10 mm) according to established bacteriological criteria [17].

Data Analysis

Data are expressed as mean ± SD. Cream preparation evaluation results were calculated in triplicate and averaged. Antibacterial activity data were expressed as inhibition zone diameters in millimetres from each replicate, with inhibition categories determined accordingly.

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