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Formulation and Stability Test of Herbal Clay Mask Ethanol Extract of Rambutan Leaves (*Nephelium lappaceum* L.) and Antibacterial Activity Test Against *Propionibacterium acnes*

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Abstract

Acne is a common skin problem caused by increased sebum production and colonization by the bacterium *Propionibacterium acnes*. Long-term use of antibiotics can trigger bacterial resistance, making natural-based treatment alternatives necessary. Rambutan leaves (*Nephelium lappaceum L.*) are known to contain secondary metabolites such as flavonoids, tannins, saponins, and alkaloids that have potential antibacterial properties. This study aimed to evaluate the effect of varying concentrations of kaolin and bentonite on the physical characteristics, stability, and antibacterial activity of an ethanol extract-based rambutan leaf clay mask formulation against *Propionibacterium acnes*. Extraction was performed using the maceration method with 96% ethanol. The clay mask formulations were prepared in three variations (F1, F2, F3) with different ratios of kaolin and bentonite, and then tested for physical characteristics, stability using a cycling test, and antibacterial activity via the well diffusion method. The results showed that variations in kaolin and bentonite concentrations affected the physical properties of the formulations, where an increase in kaolin increased viscosity and adhesion but decreased spreadability. Stability testing indicated that Formula 2 exhibited the best stability with no significant changes during the cycling test, both pharmaceutically and statistically, as demonstrated by Friedman test results with a p-value > 0.05 for each test parameter. All formulations exhibited moderate antibacterial activity against *Propionibacterium acnes*, with the largest inhibition zone observed in Formula 1 (8.38 ± 0.19 mm). Thus, rambutan leaf extract has the potential to be developed as an active ingredient in an anti acne clay mask formulation, with Formula 2 being the most stable formulation.

Keywords: *Nephelium lappaceum* L.; clay mask; kaolin; bentonite; *Propionibacterium acnes;* antibacterial.

INTRODUCTION

Facial appearance is a crucial aspect related to self-confidence, prompting many individuals to undergo various skin care treatments, whether through beauty clinics or the use of skin *care* products. However, improper product selection can lead to various skin issues, one of which is acne (Kusumastuti & Sasi, 2023). Acne is an inflammatory skin condition caused by excessive sebum production, which leads to blockages in the sebaceous glands. These blockages can interact with bacteria such as *Propionibacterium acnes*, thereby triggering the formation of acne lesions (Lavhe & Khandare, 2021). Globally, acne (*Acne vulgaris*) is one of the most common skin diseases, affecting more than 500 million people. In Indonesia, the prevalence of acne is quite high, particularly among adolescents aged 15–18 years. Hormonal changes during puberty play a role in increasing sebum production, thereby raising the risk of acne. Therefore, acne is one of the skin conditions requiring effective and sustained management (Fadilah, 2021).

Acne treatment generally involves antibiotics such as erythromycin, clindamycin, and tetracycline, administered either orally or topically. However, long-term antibiotic use has the potential to cause bacterial resistance, particularly in *Propionibacterium acnes*. High levels of resistance to several antibiotics have been reported in various countries, including Indonesia, which can reduce the effectiveness of therapy (Islam et al., 2023). This situation underscores the need to develop safer and more effective treatment alternatives, one of which involves the use of natural ingredients. The rambutan plant (*Nephelium lappaceum L.*) is known to contain secondary metabolites such as flavonoids, tannins, saponins, and alkaloids that exhibit antibacterial activity. Therefore, rambutan leaf extract has the potential to be developed as an active ingredient in topical formulations to inhibit the growth of acne-causing bacteria (Putri et al., 2021).

In addition to the active ingredient, the formulation also affects therapeutic effectiveness. Clay mask are a type of formulation with high adsorption capacity for excess oil, dirt, and pollutant particles on the skin. Mineral components such as kaolin and bentonite play a crucial role in enhancing these capabilities. Kaolin gently cleanses the skin and improves user comfort, while bentonite has a stronger ability to absorb oil and impurities and helps unclog blocked pores. The combination of both has the potential to produce an optimal formulation for oily and acne-prone skin (Fauziah et al., 2022).

Based on the above, this study aimed to analyze the effect of varying concentrations of kaolin and bentonite on the physical characteristics and stability of a clay mask formulation containing ethanol extract of rambutan leaves (*Nephelium lappaceum L.*), as well as to determine its antibacterial activity against *Propionibacterium acnes* based on the resulting inhibition zone values.

METHODS

Research Location

Plant identification was conducted at the Plant Taxonomy Laboratory, Department of Biology, FMIPA, Padjadjaran University to confirm the species used in the study. Subsequently, the extraction, phytochemical screening, formulation, and activity testing stages were carried out at the Pharmaceutical Laboratory of Perjuangan University, Tasikmalaya, following applicable laboratory procedures.

Processing of Raw Materials

Samples of rambutan leaves (*Nephelium lappaceum* L.) were collected directly from plants in Warung Buah Hamlet, Neglasari Village, Banjar Subdistrict, Banjar City. The leaves used were taken from the middle to upper parts of the branches, excluding young shoots or yellowing older leaves, characterized by a dark green color, an elliptical shape, a pointed tip, and a blunt base. The leaves were whole, undamaged, and free of spots, with pinnate venation, a regular leaf vein arrangement, and a relatively smooth surface. They were then placed in a collection container and weighed (Kiko et al., 2023). The collected leaves were subsequently subjected to wet sorting to separate debris and damaged parts. The samples are then washed and dried by sun-drying under a black cloth cover. After drying, they are reweighed to determine the moisture loss. The crude drug is then ground into powder using a grinder, followed by sieving through a No. 60 mesh to obtain a fine powder. The resulting crude drug powder was weighed and stored in a sealed container protected from light (Tari et al., 2022).

Phytochemical Screening

This process was conducted to obtain information regarding the chemical compounds contained in rambutan leaves. The identification of chemical compound groups to be conducted includes alkaloids, saponins, tannins, flavonoids, and triterpenoids.

Formulation

The formula used in the preparation of the rambutan leaf extract clay mask is provided in Table 1.

Table 1. Clay Mask Formulation.

Ingredients	Function	Formula (%)		
		FI	FII	FIII
Rambutan Leaf Ethanol Extract *	Active ingredient	5	5	5
Kaolin **	Base	25	25.5	26
Bentonite **	Base	2	1 .5	1
Glycerin **	Humectant	2	2	2
Nipagin **	Preservative	0.1	0.1	0.1
Xanthan Gum **	Thickener	0. 8	0. 8	0. .8
Sodium Lauryl Sulfate **	Surfactant	1	1	1
Distilled water to	Solvent	100	100	100

* (Marzuki et al., 2025)
** (Pamungkas et al., 2024)

Formulation Evaluation

Several evaluations were conducted on the rambutan leaf ethanol extract clay mask formulation, including organoleptic, homogeneity, spreadability, adhesion, drying time, pH, and viscosity.

Stability Testing

Stability testing was performed using a cycling test method over 12 days (6 cycles), consisting of storage at 4°C for 24 hours and 40°C for 24 hours as one cycle. The testing included organoleptic evaluation, pH, spreadability, homogeneity, adhesion, viscosity, and drying time. Temperature variations were used to evaluate the stability of the formulation against changes in environmental conditions (Febriani et al., 2022).

Antibacterial Activity Test

Instruments were sterilized in an autoclave at 121°C for 15 minutes (Sari et al., 2024). MHA medium was prepared by dissolving 13.3 g in 350 mL of distilled

water, heating until clear, sterilizing, and then aseptically pouring into Petri dishes (Purniasih et al., 2022). A *McFarland* 0.5 solution was prepared from 1% H_2SO_4 and 1% $BaCl_2$ (Fatoni, 2023). A Propionibacterium acnes suspension was prepared in 0.9% NaCl to a *McFarland* 0.5 equivalent. The inhibition test was performed using the well diffusion method, beginning with the application of the bacterial suspension onto the medium, followed by the creation of three wells containing the sample, a positive control (1% clindamycin), and a negative control (100 µL). The plates were incubated for 24 hours at 37°C, and the inhibition zones were measured (Oktaviani et al., 2020).

Data Analysis

Data analysis was performed using descriptive methods with tables and figures, followed by statistical analysis using one-way ANOVA to determine the efficacy of the formulation and antibacterial activity against *Propionibacterium acnes* by comparing the diameters of the resulting inhibition zones.

RESULTS AND DISCUSSION

Preparation Results

Table 2. Sample Preparation Results.

Sample	Sample Wet (g)	Dry Sample (g)	Powder (g)	% Shrinkage
Rambutan leaves	4500	2000	1660	55.5

The results of the sample preparation with identification number No. 23/HB/01/2026 show a decrease in weight from the wet sample to the powdered form due to the drying process and the reduction in moisture content in the crude drug.

Extraction

Table 3. Extraction Results.

Powder (g)	Concentrated Extract (g)	Yield (%)
500	137.844	27.56

The rambutan leaf extract yield of 27.56% indicates that the extraction process proceeded well and met the general requirement of a yield >10%. This high yield suggests that a greater amount of compounds was successfully extracted from the raw material, indicating that the extraction process was effective. Next, the obtained extract underwent phytochemical screening to determine the content of secondary metabolites, the results of which are presented in Table 4.

Phytochemical Screening

Table 4. Results of Phytochemical Screening.

Compound Class	Sample		Test Results
	Dried herb powder	Extract	
Alkaloids			
1.Mayer's reagent	+	+	White precipitate
2.Wagner's reagent	+	+	Brown precipitate.
3.Dradendroff's reagent	-	-	Orange precipitate
Flavonoid	+	+	Reddish-yellow color
Tannin			
1.Gelatin	+	+	White precipitate
2. $FeCl_3$	+	+	Blue-black color
Saponin	+	+	Foam formation
Triterpenoids	-	-	Brownish-red color

Note: (+) Positive for the compound; (-) Negative for compounds.

Based on Table 4, the results of the phytochemical screening indicate that the rambutan leaf extract is positive for alkaloids (Mayer and Wagner reagents), flavonoids, tannins, and saponins, and negative for triterpenoids. These results are indicated by the formation of a precipitate or a characteristic color change in each test. Subsequently, the obtained extract was used in formulations, and the preparations were evaluated to determine their physical characteristics.

Formulation Evaluation

Table 5. Organoleptic Test of the Preparation.

Formula	Form	Color	Aroma
F1	Semi-Solid	Green	Distinctive Tea
F2	Semi-Solid	Green	Tea-like
F3	Semi-Solid	Green	Characteristic Tea



(F1) (F2) (F3)

Figure 1. Organoleptic Test.

Based on the organoleptic evaluation results in the table, formulations F1, F2, and F3 exhibit relatively similar characteristics, namely a semi-solid form, green color, and a characteristic aroma of rambutan leaf extract. This similarity indicates that variations in the base do not significantly affect the appearance of the formulation. The green color and characteristic aroma originate from the dispersed rambutan leaf extract well incorporated into the base. However, visually, there were differences in viscosity among the formulations, which were likely influenced by variations in the concentrations of bentonite and kaolin in the formulation base.

Table 6. Homogeneity Test of the Formulation.

Formulation	Homogeneity
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Based on the homogeneity test results in the table, all formulations (F1, F2, and F3) showed homogeneous results characterized by the absence of coarse particles, lumps, or phase separation. This indicates that all components of the formulation are evenly mixed. Homogeneity is important to ensure uniform distribution of the active ingredient so that the resulting effects are more consistent (Sari et al., 2024)

Table 7. Formulation pH Test.

Formula	Average ± SD pH
F1	6.12 ± 0.075
F2	6.78 ± 0.005
F3	6.86 ± 0.011

The pH test was conducted with three replicates. Differences in pH values between formulations were influenced by variations in the base composition, where an increase in kaolin concentration from F1 to F3 caused the pH of the formulation to tend to increase. This is due to the relatively neutral to slightly basic nature of kaolin, whereas bentonite can affect pH through its cation exchange properties (Rowe et al., 2019). Nevertheless, all formulations remained within a pH range close to neutral (±5.00–7.50), making them safe for topical use and unlikely to cause irritation.

Table 8. Spreadability Test of the Formulation.

Formula	Mean ± SD Spreadability (cm)
F1	6.96 ± 0.577
F2	6.36 ± 0.577
F3	6.10 ± 0.100

Spreadability testing was conducted in triplicate. The results showed that an increase in the kaolin concentration in the formulation caused the spreadability value to decrease. This was due to the increased viscosity of the preparation resulting from kaolin's properties as a thickening agent, thereby limiting the preparation's ability to spread. The higher the viscosity, the greater the force required for spreading, resulting in a smaller spread area (Elfiyani et al., 2023).

Table 9. Adhesion Test of the Preparation.

Formula	Mean ± SD Adhesion Time (Seconds)
F1	5.43 ± 0.577
F2	7.58 ± 0.339
F3	8.39 ± 0.026

Adhesion testing was conducted in three replicates. The results showed that increasing the kaolin concentration in the formulation led to longer-lasting adhesion of the formulation. This was due to an increase in the number of particles, which formed a denser structure and strengthened cohesive and adhesive interactions, thereby increasing the viscosity and adhesion of the formulation. The higher the kaolin concentration, the stronger the viscoelastic properties of the system, which support longer adhesion (Boulet et al., 2022).

Table 10. Formulation Viscosity Test.

Formula	Average ± SD Viscosity (Pa·s)
F1	17.375 ± 0.135
F2	22.155 ± 0.017
F3	26.802 ± 0.041

Viscosity testing was conducted in three replicates. The results showed that increasing the kaolin concentration in the formulation led to an increase in the viscosity of the preparation. This was due to the increased number of solid particles capable of binding water and forming a denser network structure, thereby impeding flow and increasing flow resistance. The higher

the kaolin concentration, the stronger the interactions between particles, causing the system to become thicker (Shakeel et al., 2021).

Table 11. Drying Time Test.

Formula	Average ± SD Drying Time of the Preparation (Minutes)
F1	10.32 ± 0.030
F2	14.06 ± 0.015
F3	16.02 ± 0.005

Drying time tests were conducted in three replicates. The results showed that increasing the kaolin concentration prolonged the drying time of the formulation. This was due to increased viscosity and the formation of a denser particle structure, which hindered water evaporation. Additionally, kaolin's water-absorbing properties cause water to be more tightly bound within the system, thereby slowing the evaporation process (Boulet et al., 2022).

Formulation Stability Test

Table 12. Organoleptic cycling test.

Formula	Before Cycling Test	After Cycling Test
F1	Semi-solid, green, characteristic tea odor	Semi-Solid, Green, Distinctive Tea Odor
F2	Semi-Solid, Green, Distinctive Tea Odor	Semi-Solid, Green, Distinctive Tea Scent
F3	Semi-Solid, Green, Distinctive Tea Scent	Semi-solid, green, characteristic tea scent

Based on the test results in Table 12, the organoleptic test using the cycling test method showed no visual changes from cycle 1 to cycle 6. The color, odor, and form of the preparation remained stable without phase separation. This indicates that the rambutan leaf extract clay mask preparation has good physical stability against temperature fluctuations.

Table 13. Homogeneity test using the cycling test.

Formula	Before Cycling Test	After Cycling test
F1	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous

Based on the test results in Table 13, during the cycling test the formulation remained homogeneous from cycle 1 through cycle 6, as evidenced by the absence of coarse particles or phase separation. This indicates that the formulation exhibits good physical stability against temperature changes and is capable of maintaining an even distribution of components.

Table 14. pH cycling test.

Formula	Before Cycling Test	After Cycling test
F1	6.12 ± 0.075	5.24 ± 0.005
F2	6.78 ± 0.005	6.77 ± 0.011
F3	6.86 ± 0.011	7.56 ± 0.011

Based on table 14, variations in the concentrations of kaolin and bentonite affect the pH of the clay mask formulations. Formula 1 (25% kaolin; 2% bentonite) has the lowest pH (5.24), followed by Formula 2 (25.5%; 1.5%) at 6.77, and Formula 3 (26%; 1%) at the highest value of 7.56. Changes in pH during *the* cycling test were influenced by the base composition, where bentonite, with its high *swelling* and ion-exchange capacity, tended to lower the pH, while the more inert kaolin caused the pH to increase or remain relatively stable (Herbig et al., 2023). Overall, all formulations still met the pH range for topical preparations (5.00–7.50), but Formulation 1 and Formulation 3 were close to the range limits and were therefore considered suboptimal.

Table 15. Spreadability Test (Cycling Test).

Formula	Before Cycling Test	After Cycling test
F1	6.96 cm ± 0.577	6.5 cm ± 0.028
F2	6.36 cm ± 0.577	6.0 cm ± 0.057
F3	6.10 cm ± 0.100	4.8 cm ± 0.057

Based on Table 15, variations in kaolin and bentonite concentrations affect the spreadability of the clay mask formulation. Formula 1 (25% kaolin; 2% bentonite) had the highest spreadability (6.5 cm), followed by Formula 2 (6 cm) and Formula 3 (4.8 cm). Increasing the kaolin concentration causes viscosity to rise, thereby reducing spreadability. During the cycling test, all formulations experienced a decrease in spreadability due to changes in physical structure, such as hydration–dehydration processes and particle compaction that increased viscosity. The greatest decrease occurred in Formula 3 because the higher kaolin concentration formed a more compact structure (Seifert, 2020). Nevertheless, all formulations still met the clay mask spreadability requirements, which are 5–7 cm.

Table 16. Adhesion Test (Cycling Test).

Formula	Before Cycling test	After Cycling test
F1	5.43 seconds ± 0.577	6.92 seconds ± 0.502
F2	7.58 seconds ± 0.339	8.43 seconds ± 0.035
F3	8.39 seconds ± 0.026	11.11 seconds ± 0.585

Based on Table 16, variations in kaolin and bentonite concentrations affect the adhesion of the clay mask formulations. Formula 1 (25% kaolin; 2% bentonite) had the lowest adhesion (6.47 seconds), followed by Formula 2 (7.92 seconds) and Formula 3 (9.48 seconds). Increasing the kaolin concentration results in longer adhesion time due to its adsorbent properties that

enhance adhesion, whereas bentonite, which *swells,* tends to form a stiffer structure, resulting in shorter adhesion time (Kamila, 2021). During the cycling test, adhesion time increased due to structural compaction and increased viscosity caused by the repeated hydration dehydration process. Pharmaceutically, all formulations met the adhesion requirement (>4 seconds).

Table 17. Viscosity Test (Cycling Test).

Formula	Before Cycling test	After Cycling test
F1	17.375 P.as ± 0.135	21.379 P.as ± 0.041
F2	22.155 N·m ± 0.017	22.398 P.as ± 0.161
F3	26.802 P.as ± 0.041	29.928 P.as ± 0.028

Based on Table 17, variations in kaolin and bentonite concentrations affect the viscosity of the clay mask formulations. Formula 1 (25% kaolin; 2% bentonite) has the lowest viscosity (21,379 Pa·s), followed by Formula 2 (22,398 Pa·s) and Formula 3 (29,928 Pa·s). An increase in kaolin concentration causes viscosity to risedue to the formation of a denser and stronger particle structure (Shakeel et al., 2021). During the cycling test, viscosity tended to increase due to particle reorganization and structural compaction caused by repeated temperature changes, as well as network strengthening in the clay system (Badruddoza et al., 2023). Pharmaceutically, all formulations met the viscosity requirements (4–40 Pa·s).

Table 18. Drying Time Test (Cycling Test).

Formula	Before Cycling Test	After Cycling Test
F1	10.32 minutes ± 0.030	11.15 minutes ± 0.062
F2	14.06 minutes ± 0.015	14.11 minutes ± 0.015
F3	16.02 minutes ± 0.005	16.56 minutes ± 0.005

Based on Table 18, variations in kaolin and bentonite concentrations significantly affected the drying time of the clay mask formulations. Formula 1 (25% kaolin; 2% bentonite) had the fastest drying time (11.15 minutes), followed by Formula 2 (14.11 minutes) and Formula 3 (16.56 minutes). An increase in kaolin concentration results in a longer drying time due to the formation of a denser layer, which inhibits water diffusion and evaporation. During the cycling test, drying time tended to increase due to structural compaction and increased viscosity, which slowed water movement (Ruggeri et al., 2023). From a pharmaceutical standpoint, all formulations met the drying time requirements (10–25 minutes).

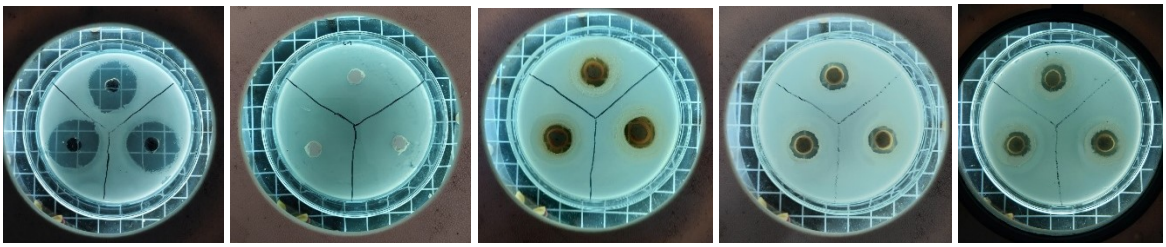
All data from the cycling test were statistically analyzed using the Friedman test because they did not meet the assumption of normality and therefore could not be analyzed using ANOVA. The results of the analysis showed that Formula 2 had a p-value > 0.05 for all test parameters, indicating no significant differences during storage. This suggests that Formula 2 has the best physical stability compared to the other formulas. Therefore, the base in Formula 2 was selected and used as the negative control in the antibacterial activity test.

Antibacterial Activity Test

Table 19. Antibacterial Activity Test.

Sample	Replication (mm)			Mean ± SD (mm)	Category
	1	2	3		
F1	8.6	8.23	8.32	8.38 ± 0.19	Moderate
F2	7.41	8.42	7.15	7.66 ± 0.67	Moderate
F3	6.66	5.9	6.27	6.27 ± 0.38	Moderate
Control +	19.82	20.62	19.28	19.90 ± 0.67	Strong
Control -	0	0	0	0	-

Note: ≤ 5 mm = weak, 5–10 mm = moderate, 11–20 mm = strong, >20 mm = very strong.



(a) (b) (c) (d) (e)

Figure 2. (a) Clindamycin Positive Control (b) Negative Control (c) Formula 1 (d) Formula 2 (e) Formula 3

Based on Table 19, all formulations exhibited inhibition zones, except for F0 (based on Formula 2), which showed no antibacterial activity. Formula 1 and Formula 2 had an average inhibition zone of 8.38 mm,

while Formula 3 had an average of 6.27 mm, all of which fall into the moderate category. This antibacterial activity is believed to originate from the secondary metabolites in rambutan leaf extract, such as flavonoids, alkaloids, saponins, and tannins, which act by damaging cell membranes, inhibiting cell wall synthesis, and disrupting bacterial metabolism (Ramadhani et al., 2024). Additionally, increasing kaolin concentration and decreasing bentonite concentration tend to reduce the inhibition zone. This is due to kaolin's adsorbent properties, which can bind active ingredients and increase the formulation's viscosity, thereby inhibiting the diffusion of active ingredients into the medium and reducing antibacterial activity (Ruggeri et al., 2023).

Data Analysis

In the stability testing, a non-parametric test was conducted using the Friedman test because the data obtained did not meet the assumption of normality (p-value < 0.05). After conducting the Friedman test, it can be seen that Formula 2 has a significance value (p-value > 0.05) for various test parameters. This indicates that there were no significant differences during the testing cycle, meaning Formula 2 exhibits good stability across all observed parameters. Conversely, Formula 1 and Formula 3 showed variations or changes in some parameters, resulting in relatively lower stability compared to Formula 2.

In the bacterial activity test, based on the results of statistical analysis, the data met the assumptions of normality and homogeneity, making it appropriate to conduct an ANOVA test. The results of the ANOVA test showed significant differences among the samples tested ($p < 0.05$).

CONCLUSION

Based on the results of the study, it can be concluded that variations in the concentrations of kaolin and bentonite affect the physical properties and stability of the rambutan leaf (*Nephelium lappaceum* L.) ethanol extract clay mask formulation. Increasing the kaolin concentration tends to increase viscosity and adhesion, but decreases spreadability. Among the tested formulations, formulation 2 showed the best stability as it did not undergo significant changes in parameters during storage. Additionally, the rambutan leaf ethanol extract clay mask formulation also exhibited antibacterial activity against Propionibacterium acnes, as indicated by the formation of inhibition zones in all formulations, with the highest activity demonstrated by formulation 1.

Competing Interests: The authors declare that there are no competing interests.

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