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Evaluation of irritation and delayed hypersensitivity potential of a *Clinacanthus nutans* cream: A phase 1 clinical trial

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ABSTRACT

Clinacanthus nutans (CN), commonly known as Sabah snake grass, is traditionally used in Malaysia for burns, bites, viral lesions (e.g., Varicella Zoster), diabetes, gout, and other inflammatory skin conditions. Recent studies have demonstrated CN's anti-inflammatory, antiviral, antioxidant, anticancer, antidiabetic, and immunomodulatory effects. Despite promising preclinical data, human safety data on topical CN formulations remain limited. A single-centre clinical trial was conducted at Hospital Al-Sultan Abdullah (HASA), Universiti Teknologi MARA (UiTM), from July to December 2024 to evaluate the safety and tolerability of a topical CN cream in healthy adults (≥18 years). Exclusion criteria included pregnancy, active skin disease, recent topical/systemic therapy, or known allergy to CN or chlorophyll. Following ISO 10993-10:2002 standards, participants underwent patch testing at 48, 96 hours, and Day 7 for irritation and allergy, followed by a 21-day occlusive application for sensitisation and tolerability, and photoallergic/phototoxicity evaluation comparing ultra violet A (UVA)-irradiated (5 J/cm²) and non-irradiated sites, with readings at 48, 72 hours, and one week. Ninety-two participants were enrolled (66.3 % female). Three (3.3 %) developed mild allergic contact dermatitis, and one (1.1 %) showed intolerance during prolonged exposure. No photoallergic or phototoxic responses were observed. All adverse events were mild, reversible, and occurred only in female participants; three were obese and one was overweight. Topical CN cream demonstrated good tolerability, with no photo-related reactions, fulfilling international safety benchmarks for botanical topical preparations. These findings justify further clinical investigation and development for potential therapeutic use.

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1. INTRODUCTION

Clinacanthus nutans (CN), commonly known as Sabah snake grass, is a medicinal plant native to Malaysia, particularly Sabah, where it is locally referred to as *Daun Belalai Gajah*. Traditionally, CN has been used for a wide range of therapeutic purposes, including the topical treatment of burns, insect and snake bites, and lesions caused by the Varicella zoster virus as well as oral administration for metabolic disorders such as diabetes and gout (Lin et al., 2023; Sabindo et al., 2024; Alam et al., 2016).

In recent years, extensive pharmacological investigations have highlighted the diverse bioactive potential of CN, encompassing anti-inflammatory, antiviral, antioxidant, anticancer, antidiabetic, and immunomodulatory properties (Khoo et al., 2018; Lin et al., 2021; Ng et al., 2022). *CN* has been reported to possess cosmeceutical potential and antioxidant-related skin benefits, although direct evidence for anti-tyrosinase-mediated skin-whitening activity remains limited (Kamarudin et al., 2017). Both in vitro and in vivo studies have further demonstrated its anticancer effects against various human cancer cell lines, including D24 and MM418C1 melanoma as well as A431 squamous cell carcinoma lines (Fong et al., 2020; Marzuki et al., 2024).

Clinically, topical formulations of CN have produced encouraging results in treating localised viral and bacterial skin infections. A systematic review and meta-analysis of randomised clinical trials have reported significant efficacy of CN cream in managing genital herpes simplex and herpes zoster infections (Kongkaew et al., 2011 CN cream has also demonstrated therapeutic potential for *condylomata acuminata*, achieving a median clearance rate of 82% compared with 97% for podophyllin in low-risk HPV patients after 4 weeks of treatment (Jiamton et al., 2022). In addition, CN exhibits antibacterial activity against Staphylococcus aureus and antiviral effects in cutaneous infections (Limpanich et al., 2025; Alam et al., 2016).

The cytotoxic and antiproliferative effects of CN's secondary metabolites, particularly chlorophyll derivatives, and their potential to generate reactive oxygen species (ROS) under phototherapeutic conditions remain largely unexplored. These mechanisms may hold therapeutic promise for the management of premalignant lesions such as actinic keratosis (AK). The chlorophyll-rich secondary metabolites of CN can modulate oxidative stress and inflammatory pathways, supporting its potential role in photoactive therapy (Ng et al., 2022; Sarjadi et al., 2018; Zainol et al., 2019). Chlorophyll-derived compounds such as pheophytins, pheophorbide derivatives, chlorophyllides, and related chlorophyll catabolites/phyllobilins have been described in chlorophyll degradation pathways and may contribute to photoactive biological effects (Kuai et al., 2018; Maroneze et al., 2019; Xodo et al., 2012). More generally, photodynamic therapy can induce reactive oxygen species–mediated cellular injury, cell-cycle arrest, and apoptosis in cancer cells, while recent CN-specific photocytotoxicity findings suggest that CN leaf extracts may warrant further investigation as a natural photosensitising source (Chen et al., 2024).

In a previous study, ethanolic extracts of CN at 5 μg/mL, combined with 5 minutes of phototherapy (17.04 J/cm^2), produced significant antiproliferative effects both in vitro and in vivo in A431 squamous cell carcinoma lines (Marzuki et al., 2024). The increase in intracellular ROS levels was inversely correlated with cell viability under light irradiation, with a 1.3-fold rise in ROS generation in 5 μg/mL CN-treated A431 cells subjected to photodynamic therapy compared to non-irradiated controls. Similar mechanisms have been described in photosensitiser-mediated photodynamic therapy, where ROS initiate oxidative chain

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reactions targeting deoxyribonucleic acid (DNA), proteins, and lipids, leading to irreversible cellular injury and apoptosis (Kwiatkowski et al., 2018; Robertson et al., 2009).

This study was designed to evaluate the cutaneous safety profile of a topical CN formulation in terms of its allergy, irritation and sensitization potential. The phototoxicity and photoallergy potentials were also evaluated.

2. MATERIAL & METHODOLOGY

2.1 Study design

This single-centre clinical trial was conducted at the Dermatology Clinic, Hospital Al-Sultan Abdullah (HASA), also known as Hospital Universiti Teknologi MARA (UiTM), Puncak Alam. All eligible participants were screened, examined, and recruited between July and December 2024. Participants were enrolled through convenience sampling based on availability and willingness to participate.

The sample-size calculation followed the method described by Charuwichitratana et al. (1996). Assuming a 15% prevalence of the factor of interest in the population, with 8% absolute precision at a 95% confidence level and allowing for a 20% dropout rate, the required sample size was 92 participants.

Healthy volunteers aged 18 years and above were eligible for inclusion. Exclusion criteria included pregnancy or breastfeeding, skin diseases (e.g. psoriasis, eczema, vitiligo), active cutaneous infection, initiation of new skin care or phototherapy within the preceding three weeks, current use of systemic corticosteroids or antihistamines, and known allergy to chlorophyll or chlorophyll-containing products, planned pregnancy during the study period and participant-related factors that could affect compliance with study procedures, such as significant psychiatric illness, substance abuse, or inability to attend scheduled visits.

2.2 Investigational product

The investigational product (IP) evaluated in this study was a topical cream containing a standardized extract derived from CN. The active component of the standardized extract was identified as a chlorophyll catabolite, with a dosage concentration of 5 μg/mL in the finished formulation. The other ingredients are tetrasodium EDTA, hydroxyethyl acrylate, C10-30 alkyl acrylate, glyceryl stearate, PEG-00 stearate, cetearyl alcohol, ceteareth-20, dimethicone, isononyl isononanoate, phenoxylethanol, ethylhexyglycerin, triethanolamine, chlorophyllin, aqua, glycerin and petrolatum. All investigational cream samples were manufactured by Skinology Laboratories Sdn. Bhd. (Malaysia).

No prior animal studies evaluating irritation, sensitisation, or photo-safety were available for this formulation. However, the cream is commercially available and registered as a cosmetic product with the National Pharmaceutical Regulatory Agency (NPRA) under the Ministry of Health Malaysia, in compliance with the Control of Drugs and Cosmetics Regulations 1984. The product is marketed for skin barrier improvement, with claims related to the repair and soothing of damaged or sensitive skin.

The safety and tolerability of the topical CN cream were evaluated using a standardised patch-test protocol adapted from internationally recognised guidelines—including ISO 10993-10:2002 (Biological Evaluation of Medical Devices – Part 10: Tests for Irritation and Delayed-Type Hypersensitivity) (International Organization for Standardization, 2002) and the European Society of Contact Dermatitis

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(ESCD) Guideline for Diagnostic Patch Testing (Johansen et al., 2015)—to ensure methodological consistency and international comparability. The study was conducted in two phases to evaluate the cutaneous safety, tolerability, and photoreactivity of the topical CN cream.

- Phase 1 (Allergy, Irritation, Sensitisation and tolerability assessment): A standard occlusive patch test with investigational product CN cream was performed on all participants. Individuals showing no allergic or irritant responses proceeded to Stage 2, which involved a 21-day daily application to assess cumulative tolerability.
- Phase 2 (Photoallergic and phototoxicity assessment): Photoallergic and phototoxic responses were assessed by comparing reactions between non-irradiated and ultra violet A (UVA)-irradiated sites.

2.3 Study methodology

The total sample of 92 participants was divided into two groups. In Phase 1, 46 participants underwent allergy, irritation, sensitisation, and 21-day tolerability assessments. In Phase 2, a separate group of 46 participants underwent photoallergy and phototoxicity assessments. This allocation was designed to reduce participant burden, limit cumulative exposure to the investigational product, and enable completion of both safety components within the approved clinical workflow.

A hypoallergenic IQ Ultra™ chamber containing 0.25 g of CN cream was applied to and affixed to the designated test site. All patch-test readings and clinical reactions in both trials were graded according to the International Contact Dermatitis Research Group (ICDRG) scoring system, as recommended in the European Society of Contact Dermatitis guideline for diagnostic patch testing (Johansen et al., 2015).

Phase 1: Allergy, Irritation, Sensitisation, and Tolerability Assessment

Stage 1 – Patch Test for Allergy, Irritation, and Sensitisation Assessment

Participants underwent a standard occlusive patch test to detect possible allergic, irritant, or sensitisation reactions. Each chamber containing 0.25 g of CN cream was affixed to the upper arm by the investigator. Readings were obtained at 48 hours (Day 2), 96 hours (Day 4), and Day 7 to identify early and delayed hypersensitivity responses.

Stage 2 – 21-Day Cumulative Tolerability Assessment

Participants who exhibited no allergic or irritant responses proceeded to the 21-day tolerability test. The CN cream was applied once daily to the contralateral arm under occlusion. Skin assessments were conducted on Days 7, 14, and 21. Participants who developed cutaneous reactions discontinued from further applications, while those who completed 21 days without reactions were considered to have acceptable tolerability.

Phase 2: Photoallergic and Phototoxicity Assessment

Photoallergic and phototoxic evaluations were conducted to determine cutaneous responses to UVA exposure following CN application.

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At baseline (Day 0), two sets of CN cream patches (0.25 g each) were applied separately to the right and left upper arms by investigator, with one site designated for UVA irradiation and the contralateral site serving as a non-irradiated control. After 24 hours (Day 1), the irradiated site was exposed to UVA light at a total dose of 5 J/cm² for 6 minutes 8 seconds, using a device with an output intensity of 13.6 mW/cm².

Follow-up assessments were performed on Day 3, Day 4, and Day 8, corresponding to 48, 72, and 168 hours post-irradiation, respectively. Cutaneous reactions were evaluated for erythema, oedema, vesiculation, and pigmentation at both irradiated and non-irradiated sites.

Interpretation of test results was based on site-specific reactions:

- A positive reaction only at the irradiated site suggested a photoallergic or phototoxic response.
- A comparable positive reaction at irradiated and non-irradiated site's reaction indicated allergic or irritant contact dermatitis.

2.4 Statistical analysis

Data were cleaned and summarized using SPSS version 24.0 and STATA version 19.0. The distribution of continuous variables was checked using skewness, kurtosis and histogram. Continuous variables were presented as mean and standard deviation as the data were observed to be normally distributed, while categorical variables were presented as frequency and percentage.

The association between demographics characteristics and presence of allergic reaction was tested using independent sample t test and Fisher Exact test. The 95% confidence interval of the multinomial proportions were generated using 'mpci' package available in STATA. All the tests were two sided and statistical significance was denoted by $p < 0.05$.

3. RESULTS

A total of 92 healthy volunteers were enrolled in this Phase I clinical trial. Of the 92 participants, 61 (66.3%) were female, and all were of Malay ethnicity. The mean age was 24.0 ± 4.6 years, and the mean body mass index (BMI) was 25.1 ± 5.5 kg/m². Five participants (5.4%) were current smokers. A history of atopy was reported in 31 participants (33.7%), including allergic rhinitis (n = 16), atopic dermatitis (n = 8), bronchial asthma (n = 6), and combined rhinitis with asthma (n = 1). Two participants (2.2%) reported previous contact dermatitis to alcohol-based hand rubs or detergents, and another two (2.2%) had chronic urticaria. Food allergies were documented in 9 participants (9.8%), most commonly to seafood, followed by eggs, peanuts, durian, and salak fruit. Drug allergies were reported in 5 participants (5.4%), mainly to paracetamol, mefenamic acid, penicillin derivatives, and sulphur-containing medications. Other comorbidities included glucose-6-phosphate dehydrogenase (G6PD) deficiency (n = 2), hyperthyroidism (n = 2), and scoliosis (n = 2). Participant characteristics were summarized in Table 1.

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Table 1: Characteristics of the study participants.

Variables	Participants in Allergic, irritant and tolerability assessments (n = 46)	Participants in Photoallergic and phototoxicity assessments (n = 46)	Overall (n = 92)
Age, years, mean (SD)	25.18 (5.61)	22.87 (2.97)	24.02 (4.74)
Gender, n (%)			
Male	18 (39.1)	13 (28.3)	31 (33.7)
Female	28 (60.9)	33 (71.7)	61 (66.3)
Ethnicity, n (%)			
Malay	46 (100)	46 (100)	92 (100)
Education level, n (%)			
Tertiary:	46 (100.0)	46 (100.0)	92 (100.0)
Degree	46 (100.0)	45 (97.8)	91 (98.9)
Master	0 (0.0)	1 (2.2)	1 (1.1)
Smoking status, n (%)			
No	43 (93.5)	44 (95.7)	87 (94.6)
Yes	3 (6.5)	2 (4.3)	5 (5.4)
Household monthly income, n (%)			
No income	20 (43.5)	25 (54.3)	45 (48.9)
< RM 4 850	7 (15.2)	4 (8.7)	11 (12.0)
RM 4 850 – 10 959	15 (32.6)	11 (23.9)	26 (28.3)
≥ RM 10 960	4 (8.7)	6 (13.0)	10 (10.9)
Anthropometric measurements, mean (SD)			
Height (cm)	163.61 (10.26)	160.36 (8.32)	161.98 (9.43)
Weight (kg)	73.60 (16.57)	57.41 (9.97)	65.51 (15.85)
BMI	27.75 (6.17)	21.54 (2.98)	25.09 (5.50)
Atopy, n (%)	14 (30.4)	17 (37.0)	31 (33.7)
Allergic rhinitis	10	6	16
Atopic dermatitis	0	8	8
Bronchial asthma	3	3	6
Allergic rhinitis + bronchial asthma	1	0	1
Chronic urticaria, n (%)	1 (2.2)	1 (2.2)	2 (2.2)
Contact dermatitis, n (%)	1 (2.2)	1 (2.2)	2 (2.2)
Alcohol rub	1	0	1
Detergent	0	1	1
Food allergic, n (%)	2 (8.7)	7 (15.2)	9 (9.8)
Seafood	2	4	6
Eggs	0	1	1
Peanut	0	1	1
Durian	0	1	1
Salak fruit	0	1	1
Drug allergy, n (%)	1 (2.2)	4 (8.7)	5 (5.4)
Paracetamol	1	0	1
Mefenamic acid	0	1	1
Penicillin group	0	2	2
Sulphur-containing drugs	0	1	1
Other Comorbid, n (%)	5 (10.9	2 (4.3)	7 (7.6)
G6PD	1	1	2
Hyperthyroidism	2	0	2
Scoliosis	1	1	2

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Allergy, Irritation, Sensitisation, Tolerability, and Photoreactivity

46 participants underwent testing. Three participants developed allergic contact dermatitis to CN cream between Days 5 and 7 of the standard patch tests. Two participants, 66.7% (2/3) had well-defined erythema (Grade 1), while one, 33.3% (1/3) developed pruritic well-defined erythema with papulovesicular lesions (Grade 2). All reactions resolved within 3–5 days after discontinuation of the cream and topical corticosteroid treatment (Fig. 1).

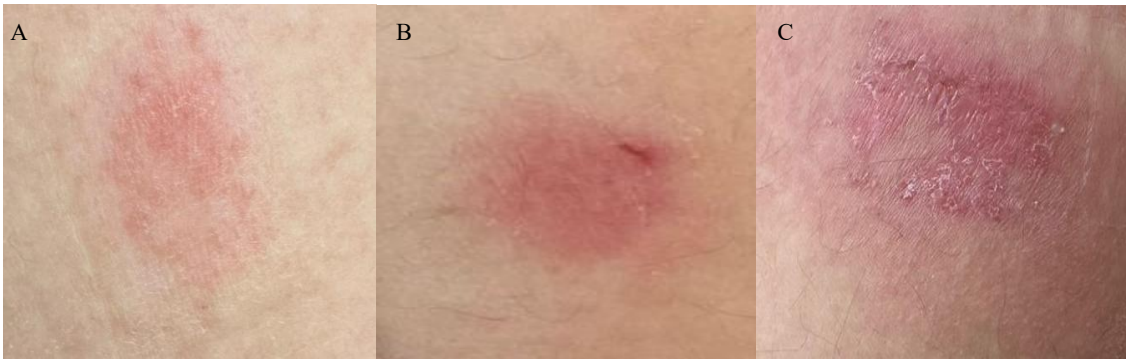

Fig 1. Cutaneous reactions observed in 2 participants between days 5 – 7 of patch test. Erythema with scale (Grade 1 reaction) observed in one participant, Figure 1A and 1B. Erythema with vesicles and scale (Grade 2 reaction) observed in another participant, Figure 1C

These three participants were excluded from the subsequent 21-day tolerability phase. Among the remaining 43 participants, one developed a reaction on Day 11, characterised by pruritic well-defined erythema, oedema, and papulovesicular eruption (Grade 2), which resolved after treatment discontinuation and mild topical corticosteroid therapy (Fig. 2).

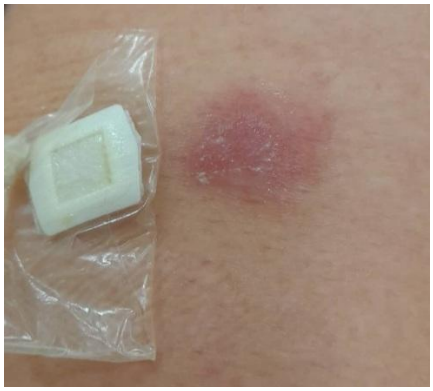

Fig 2. Cutaneous reaction observed during the 21-day test showing well-defined erythema, oedema, and papulovesicular lesions (Grade 2 reaction).

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Table 2. Factors associated with allergic reactions to CN cream in the phase 1 allergy, irritation, sensitisation, and tolerability assessment.

Characteristics	Allergic reaction				P value
	No		Yes		
	N=42		N=4		
	N *	% (95% CI)	N *	% (95% CI)	
Age, years, mean (SD)		25.25 (5.43)		29.75 (8.27)	0.137a
Gender, n (%)					
Male	18	100.0 (82.4, 100.0)	0	0.0	0.144b
Female	24	85.7 (65.6, 95.0)	4	14.3 (5.0, 34.4)	
Ethnicity, n (%)					
Malay	42	91.3 (77.5, 97.0)	4	8.7 (3.0, 22.5)	-
Education level, n (%)					
Tertiary	42	91.3 (77.5, 97.0)	4	8.7 (3.0, 22.5)	-
Occupation, n (%)					
Student	31	96.9 (81.5, 99.5)	1	3.1 (0.5, 18.5)	0.087b
Health care workers	9	81.8 (48.1, 95.6)	2	18.2 (4.4, 52.0)	
PPK		1		1	
Staff nurse		5		0	
Medical officer		3		1	
Non-Health care workers	2	66.7 (17.5, 95.0)	1	33.3 (5.0, 82.5)	
Clerk		2		0	
CRC Coordinator		0		1	
Smoking status, n (%)					
No	39	90.7 (76.1, 96.8)	4	9.3 (3.3, 23.9)	>0.950b
Yes	3	100.0 (43.9, 100.0)	0	0.0	
Household monthly income, n (%)					
No income	19	95.0 (72.7, 99.3)	1	5.0 (0.7, 27.3)	0.435b
< RM 4 850	7	100.0 (64.6, 100.0)	0	0.0	
RM 4 850 – 10 959	13	86.7 (58.1, 96.8)	2	13.3 (3.2, 41.9)	
≥ RM 10 960	3	75.0 (25.9, 96.3)	1	25.0 (3.7, 74.1)	
Anthropometric measurements, mean (SD)					
Height (cm)		164.24 (10.46)		157.00 (4.55)	0.181a
Weight (kg)		72.80 (16.74)		82.00 (13.64)	0.294a
BMI (kg/m2)		27.22 (5.89)		33.33 (7.22)	0.058a
Underweight (<18.5)	1	100.0 (20.7, 100.0)	0	0.0	0.465b
Normal (18.5-22.9)	10	100.0 (72.3, 100.0)	0	0.0	
Pre-obese (Overweight) (23.0-27.4)	13	92.9 (64.1, 99.0)	1	7.1 (1.1, 35.9)	
Obese I (27.5-32.4)	11	91.7 (60.0, 98.8)	1	8.3 (1.2, 40.0)	
Obese II (32.5-37.4)	4	80.0 (32.9, 97.0)	1	20.0 (3.0, 67.1)	
Obese III (≥37.5)	3	75.0 (25.9, 96.3)	1	25.0 (3.7, 74.1)	
Atopy, n (%)					
No	29	90.6 (73.0, 97.2)	3	9.4 (2.8, 27.0)	>0.950b
Yes	13	92.9 (64.1, 99.0)	1	7.1 (1.1, 35.9)	
Allergic rhinitis		9		1	
Bronchial Asthma		3		0	
More than 1 atopic disease		1		0	
Chronic urticaria, n (%)					
No	41	91.1 (77.1, 96.9)	4	8.9 (3.1, 22.9)	>0.950b
Yes	1	100.0 (20.7, 100.0)	0	0.0	
Contact dermatitis, n (%)					
No	41	91.1 (77.1, 96.9)	4	8.9 (3.1, 22.9)	>0.950b
Yes	1	100.0 (20.7, 100.0)	0	0.0	
Food allergy, n (%)					
No	40	90.9 (76.6, 96.8)	4	9.1 (3.2	>0.950b
Yes	2	100.0 (34.2, 100.0)	0	0.0	
Drug allergy, n (%)					
No	41	91.1 (77.1, 96.9)	4	8.9 (3.1, 22.9)	>0.950b
Yes	1	100.0 (20.7, 100.0)	0	0.0	
Other Comorbid, n (%)					
No	40	90.9 (76.6, 96.8)	4	9.1 (3.2, 23.4)	>0.950b
Yes	2	100.0 (34.2, 100.0)	0	0.0	

*Otherwise as indicated; [a]Independent sample t test; [b]Fisher Exact test

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A separate group of 46 participants underwent evaluation for photoallergic and phototoxic reactions. No adverse skin reactions were observed at either the non-irradiated or UVA-irradiated sites.

All allergic reactions occurred in female participants, corresponding to a reaction rate of 14.3% among women (95% CI: 5.0–34.4). Three affected participants were obese with BMI ≥ 27.5 kg/m^2 and one was overweight with BMI range 23-27.4 kg/m^2. Although higher reaction rates were observed in higher BMI categories, no statistically significant associations were identified between demographic or medical characteristics and the occurrence of allergic reactions ($p > 0.05$, Table 2). Only one affected participant had a history of allergic rhinitis.

Participants with G6PD deficiency and thyroid disorders were clinically stable at enrolment. As the investigational product was applied topically in a small amount under controlled study conditions. No adverse reactions, systemic symptoms, haemolytic features, or worsening of thyroid-related symptoms were observed in these participants throughout the study period.

Overall safety outcomes

In total, four participants (4.3%) experienced mild, self-limiting cutaneous reactions: three (3.3%) with allergic contact dermatitis and one (1.1%) with delayed allergic reaction (Figure 3). All affected participants were female; three were obese, and one was overweight. Only one had a history of allergic rhinitis, while none had prior food or drug hypersensitivity. No photoallergic or phototoxic responses, systemic effects, or serious adverse events were observed.

4. DISCUSSION

This Phase I clinical trial evaluated the cutaneous safety of a topical CN cream in healthy volunteers. The formulation demonstrated a favourable safety profile, with a low overall incidence of allergic contact dermatitis and irritation potential. All reactions were mild, self-limiting, and resolved within three days following short-term topical corticosteroid therapy. No photoallergic or phototoxic reactions were observed, indicating that CN cream did not induce photosensitivity under UVA exposure. Although Fitzpatrick skin phototype was not prospectively recorded, all participants were Malay; based on clinical observation, the majority were likely Fitzpatrick skin phototypes III–IV, with a minority likely phototype II. The predominance of skin phototypes III–IV may explain the absence of phototoxic responses, as higher melanin content provides greater natural photoprotection by absorbing and scattering ultraviolet radiation and reducing UV-induced oxidative injury. Melanin may therefore attenuate erythemal responses and limit visible inflammatory changes following UVA exposure. However, this interpretation should be made cautiously, as photoallergic reactions are immunologically mediated and may still occur across all skin phototypes. The absence of both phototoxic and photoallergic reactions in this cohort therefore supports the preliminary photobiologic tolerability of CN cream, while further evaluation in participants with prospectively documented Fitzpatrick skin phototypes, including lighter phototypes and patients with sun-damaged skin, is warranted.

These findings are consistent with the low frequency of adverse cutaneous reactions reported for botanically derived topical products, particularly when reactions are mild, transient, and reversible (Corazza et al., 2014; Gilissen et al., 2018). Mild irritation or sensitisation rates for standard topical vehicles or corticosteroid creams range from 2 - 7% among healthy volunteers, while contact allergy to corticosteroids occurs in approximately 0.2 - 5% of patch-tested individuals (Mahajan et al., 2024). The CN cream's overall

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4.3% reaction rate, absence of erosive or ulcerative lesions, and full recovery in all cases collectively support its excellent dermatologic and photobiologic tolerability.

The demographic profile of our study participants who developed reactions aligns with previous epidemiologic data showing that female sex, obesity, and atopy increase susceptibility to contact dermatitis (Tramontana et al., 2023; Shang et al., 2024; Yew et al., 2023). Women exhibit higher contact-sensitisation rates due to greater exposure to cosmetics and occupational allergens. In our cohort, all affected participants were overweight or obese, and the mean BMI was numerically higher among those with reactions; however, this association did not reach statistical significance. Obesity is increasingly recognised as a pro-inflammatory cutaneous state, where adipokines such as leptin and TNF-α impair epidermal barrier repair, alter the skin microbiota, and delay wound healing, thereby predisposing individuals to both irritant and allergic dermatitis (Shang et al., 2024; Yew et al., 2023). Likewise, atopic individuals possess an inherent barrier dysfunction and a Th2-skewed immune response, facilitating allergen penetration and enhancing T-cell–mediated sensitisation (Hamann et al., 2017).

Notably, the onset of all allergic reactions in this study was consistent with type IV delayed-type hypersensitivity. This pattern is typical of herbal preparations containing weak natural haptens such as flavonoids, terpenoids, and chlorophyll derivatives including pheophytins, pheophorbide derivatives, chlorophyllides, and related chlorophyll catabolites/phyllobilins (Kuai et al., 2018; Maroneze et al., 2019; Xodo et al., 2012). These compounds undergo metabolic activation and antigen presentation by Langerhans cells before eliciting clonal T-cell proliferation. Similar delayed hypersensitivity has been reported with botanical agents such as *Achillea millefolium* and *Curcuma longa* (Gilissen et al., 2018; Calapai et al., 2014). The absence of immediate erythema or burning reinforces that these reactions were allergic rather than irritant in nature.

The absence of immediate erythema, burning, or stinging further supports the interpretation that these reactions were allergic rather than irritant in nature. The absence of phototoxic and photoallergic reactions is particularly relevant given the proposed role of CN cream as a potential treatment modality for actinic keratosis (AK). Previous evidence that CN may enhance reactive oxygen species (ROS) generation during phototherapy supports its possible function as a photosensitising agent (Ismail et al., 2020; Chen et al., 2024). As photosensitisers may induce unintended phototoxic reactions in normal skin, the absence of UVA-induced erythema, exaggerated inflammation, or delayed photoallergic responses in this study provides preliminary reassurance regarding the photobiologic safety of CN cream. These findings suggest that, although CN may exert ROS-mediated antiproliferative effects under controlled phototherapeutic conditions, this activity did not translate into clinically significant photosensitivity in healthy skin. These findings support further evaluation of CN cream in patients with AK. However, these findings should be interpreted cautiously, as patients with AK often have chronically sun-damaged skin, impaired barrier function, baseline inflammation, and higher cumulative ultraviolet exposure than healthy volunteers. Therefore, future studies should evaluate CN cream in the target AK population under clinically relevant phototherapy conditions.

From a regulatory perspective, herbal topical products are considered acceptable when adverse-event frequencies remain below 5%, provided that reactions are mild, transient, and reversible (European Medicines Agency, 2016; National Pharmaceutical Regulatory Agency, 2025; US Food and Drug Administration, 2016). The present results satisfy these criteria and align with both ISO 10993-10 and the Malaysian NPRA Drug Registration Guidance Document (DRGD), which recommends confirmatory

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testing only if sensitisation rates exceed 5% (National Pharmaceutical Regulatory Agency, 2025). Accordingly, CN cream meets regulatory expectations for dermatologic safety and is suitable for further clinical investigation.

Beyond safety, this standardised chlorophyll catabolite-containing CN formulation at 5 µg/mL has reported antioxidant, anti-inflammatory, and photodynamic properties. These bioactive compounds modulate reactive oxygen species (ROS), inhibit tumour-cell proliferation, and enhance photodynamic-therapy. research (Ismail et al., 2020; Chen et al., 2024). Given the low sensitisation rate, excellent tolerance, absence of clinically detectable photoallergy or phototoxicity, and mechanistic plausibility for ROS-mediated antiproliferative activity, CN cream is a promising candidate for Phase II clinical trials targeting premalignant skin lesions such as actinic keratosis by providing a safer, plant-derived alternative with low potential for irritation.

Limitations

This study's cohort predominantly composed of young Malay adults resulting in homogeneity that limits generalizability to older age groups and ethnically diverse populations. The modest sample size, although adequate for a Phase I safety study, restricts the detection of rare adverse events. In addition, Fitzpatrick skin phototype was not prospectively recorded, which should be considered when interpreting the negative phototoxicity and photoallergy findings. Patch testing with the vehicle base and isolated CN/chlorophyll catabolite extract was not performed; therefore, the observed reactions cannot be conclusively attributed to the active botanical component alone and may also relate to excipients, preservatives, emulsifiers, or the complete formulation. Excluding participants with initial reactions from the 21-day tolerability phase may have selected for participants with more robust skin tolerance and may underestimate acceptability issues among individuals with sensitive skin. Despite these limitations, this trial provides valuable first-in-human evidence supporting the dermatologic safety and photobiologic tolerability of CN cream.

5. CONCLUSION

This Phase I study demonstrates that standardised chlorophyll catabolite-containing CN cream formulation is safe and well-tolerated when applied topically in healthy individuals. CN cream did not cause photoallergic or phototoxic reactions. All observed reactions were limited to mild, reversible dermatitis without systemic involvement. Overall, these findings are consistent with accepted safety thresholds for topical botanical formulations and support the continued clinical development of CN cream for potential therapeutic

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participant recruitment, data collection, data analysis, interpretation of findings, manuscript preparation, or the decision to submit the manuscript for publication.

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CONFLICT OF INTEREST STATEMENT

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AUTHORS' CONTRIBUTIONS

Heah Swee Kuan: Conceptualization, Data curation, Methodology, Investigation, Visualization, Data analysis, Writing – original draft. **Sabrina Ab Wahab**: Supervision, Resources, Writing – review & editing. **Tarita Taib:** Project administration, Funding acquisition.

DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

No artificial intelligence (AI) tools were used in the writing, editing, data analysis, image generation, or any other aspect of preparing this manuscript.

ETHICS STATEMENT

Ethical approval was obtained from the Research Ethics Committee Universiti Teknologi MARA (UiTM) (REC/06/2024 [ST/CT/7]). The study was conducted in accordance with the principles of the Declaration of Helsinki (World Medical Association, 2013) and the Good Clinical Practice (GCP) guidelines (International Council for Harmonisation, 2016). Written informed consent was obtained from all participants prior to enrolment.

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REFERENCES

Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A. S., Khatib, A., & Sarker, Z. I. (2016). Clinacanthus nutans: A review of the medicinal uses, pharmacology and phytochemistry. *Asian Pacific Journal of Tropical Medicine*, 9(4), 402-409. https://doi.org/10.1016/j.apjtm.2016.03.011

Calapai, G., Miroddi, M., Minciullo, P. L., Caputi, A. P., Gangemi, S., & Schmidt, R. J. (2014). Contact dermatitis as an adverse reaction to some topically used European herbal medicinal products - Part 1: Achillea millefolium-Curcuma longa. *Contact Dermatitis*, 71(1), 1-12. https://doi.org/10.1111/cod.12222

Charuwichitratana, S., Wongrattanapasson, N., Timpatanapong, P., & Bunjob, M. (1996). Herpes zoster: Treatment with *Clinacanthus nutans* cream. *International Journal of Dermatology, 35*(9), 665-666. https://doi.org/10.1111/j.1365-4362.1996.tb03699.x

Chen, C. S., et al. (2024). Phytochemical screening, antioxidant properties, and photocytotoxicity of Clinacanthus nutans leaf extracts. *Vegetos*, 37, 1652-1663. https://doi.org/10.1007/s42535-023-00731-0

Corazza, M., Borghi, A., Lauriola, M. M., Virgili, A., & Toni, G. (2014). Topical botanically derived products: Use, skin reactions, and usefulness of patch tests. A multicentre Italian study. *Contact Dermatitis*, 70(2), 90-97. https://doi.org/10.1111/cod.12124

European Medicines Agency. (2022, January 18). Guideline on quality of herbal medicinal products/traditional herbal medicinal products (EMA/HMPC/CHMP/CVMP/201116/2005 Rev. 3). https://www.ema.europa.eu/en/quality-herbal-medicinal-products-traditional-herbal-medicinal-products-scientific-guideline.

Fong, S. Y., Wimalasiri, D., Piva, T., Dekiwadia, C., Urban, S., & Huynh, T. (2019). Evaluation of cytotoxic and apoptotic activities of Clinacanthus nutans (Burm. f.) Lindau leaves against D24 human melanoma cells. *Journal of Herbal Medicine*, 17-18, Article 100285. https://doi.org/10.1016/j.hermed.2019.100285

Gilissen, L., Huygens, S., & Goossens, A. (2018). Allergic contact dermatitis caused by topical herbal remedies: Importance of patch testing with the patients' own products. *Contact Dermatitis*, 78(3), 177-184. https://doi.org/10.1111/cod.12939

Hamann, C. R., Hamann, D., Egeberg, A., Johansen, J. D., Silverberg, J., & Thyssen, J. P. (2017). Association between atopic dermatitis and contact sensitization: A systematic review and meta-analysis. *Journal of the American Academy of Dermatology*, 77(1), 70-78. https://doi.org/10.1016/j.jaad.2017.02.001

International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. (2016, November 9). Integrated addendum to ICH E6(R1): Guideline for good clinical practice E6(R2). https://database.ich.org/sites/default/files/E6_R2_Addendum.pdf

International Organization for Standardization. (2002). ISO 10993-10:2002: Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity. https://www.iso.org/standard/33364.html

Ismail, N. Z., Arsad, H., Samian, M. R., Ab Majid, A. H., Hamdan, M. R., & Mohamad, N. (2020). Antioxidant effects, antiproliferative effects, and molecular docking of Clinacanthus nutans leaf extracts. *Molecules*, 25(9), 2067. https://doi.org/10.3390/molecules25092067

Jiamton, S., Chanyachailert, P., Nanchaipruek, Y., Jantanapornchai, N., Patthamalai, P., Limphoka, P., Nokdhes, Y.-N., & Jantaravinid, J. (2022). Efficacy and safety of Clinacanthus nutans Lindau cream vs. podophyllin for the treatment of adults with condyloma acuminata. *Evidence-Based Complementary and Alternative Medicine*, 2022, Article 1577716. https://doi.org/10.1155/2022/1577716

Johansen, J. D., Aalto-Korte, K., Agner, T., Andersen, K. E., Bircher, A., Bruze, M., et al. (2015). European Society of Contact Dermatitis guideline for diagnostic patch testing. *Contact Dermatitis*, 73, 195-221. https://doi.org/10.1111/cod.12432

Johansen, J. D., Aalto-Korte, K., Agner, T., Andersen, K. E., Bircher, A., Bruze, M., Cannavó, A., Giménez-Arnau, A., Gonçalo, M., Goossens, A., John, S. M., Lidén, C., Lindberg, M., Mahler, V., Matura, M., Rustemeyer, T., Serup, J., Spiewak, R., Thyssen, J. P., & Uter, W. (2015). European Society of Contact Dermatitis guideline for

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diagnostic patch testing - recommendations on best practice. *Contact Dermatitis*, 73(4), 195-221. https://doi.org/10.1111/cod.12432

Kamarudin, M. N. A., Sarker, M. M. R., Kadir, H. A., & Ming, L. C. (2017). Ethnopharmacological uses, phytochemistry, biological activities, and therapeutic applications of Clinacanthus nutans (Burm. f.) Lindau: A comprehensive review. *Journal of Ethnopharmacology*, 206, 245-266. https://doi.org/10.1016/j.jep.2017.05.007

Khoo, L. W., Lau, C. P., Goh, B. H., et al. (2018). Pharmacological properties of Clinacanthus nutans: A systematic review. *Pharmacognosy Reviews*, 12, 27-32. https://doi.org/10.1155/2018/9276260

Kongkaew, C., Chaiyakunapruk, N., et al. (2011). Efficacy of Clinacanthus nutans extracts in patients with herpes infection: Systematic review and meta-analysis of randomised clinical trials. *Complementary Therapies in Medicine*, 19(1), 47-53. https://doi.org/10.1016/j.ctim.2010.12.003

Kuai, B., Chen, J., & Hörtensteiner, S. (2018). The biochemistry and molecular biology of chlorophyll breakdown. *Journal of Experimental Botany*, 69(4), 751-767. https://doi.org/10.1093/jxb/erx322

Kwiatkowski, S., Knap, B., Przystupski, D., Saczko, J., Kędzierska, E., Knap-Czop, K., Kotlińska, J., Michel, O., Kotowski, K., & Kulbacka, J. (2018). Photodynamic therapy-mechanisms, photosensitizers and combinations. *Biomedicine & Pharmacotherapy*, 106, 1098-1107. https://doi.org/10.1016/j.biopha.2018.07.049

Limpanich, N., Chayapakdee, P., Mekawan, K., et al. (2025). Integrative wound-healing effects of Clinacanthus nutans extract and schaftoside through anti-inflammatory, endothelial-protective and antiviral mechanisms. *International Journal of Molecular Sciences*, 26, Article 6029. https://doi.org/10.3390/ijms26136029

Lin, C. M., Chen, H. H., Lin, M. C., et al. (2021). Recent advancement in anticancer activity of Clinacanthus nutans (Burm. f.) Lindau. *Evidence-Based Complementary and Alternative Medicine*, 2021, Article 5560502. https://doi.org/10.1155/2021/5560502

Lin, C. M., Chen, H. H., Lung, C. W., & Chen, H. J. (2023). Antiviral and immunomodulatory activities of Clinacanthus nutans (Burm. f.) Lindau. *International Journal of Molecular Sciences*, 24, Article 10789. https://doi.org/10.3390/ijms241310789

Mahajan, V. K., Mehta, K. S., Chauhan, P. S., et al. (2024). Allergic contact dermatitis caused by topical corticosteroids. *Cosmoderma*, 4, Article 39. https://doi.org/10.25259/CSDM_233_2023

Maroneze, M. M., et al. (2019). Chlorophyll oxidative metabolism during phototrophic growth. *Antioxidants*, 8(12), Article 600. https://doi.org/10.3390/antiox8120600

National Pharmaceutical Regulatory Agency. (2025). Drug registration guidance document (DRGD) (3rd ed., 10th rev.). Ministry of Health Malaysia. https://www.npra.gov.my/easyarticles/images/users/1153/DRGD%20July%202025/Complete-Drug-Registration-Guidance-Document-DRGD-3rd-Edition-10th-Revision-July-2025.pdf

Ng, K. S., See, Z. Q., Tan, W. N., & Lee, L. H. (2022). Metabolomic approach for identification of antioxidative compounds from Clinacanthus nutans. *Molecules*, 27, Article 3650. https://doi.org/10.3390/molecules27123650

Robertson, C. A., Evans, D. H., & Abrahamse, H. (2009). Photodynamic therapy (PDT): A short review on cellular mechanisms and cancer research applications for PDT. *Journal of Photochemistry and Photobiology B: Biology*, 96(1), 1-8. https://doi.org/10.1016/j.jphotobiol.2009.04.001

Sabindo, N. H., Yatim, R. M., & Ponnuraj, K. T. (2024). Phytochemical composition of Clinacanthus nutans based on environmental, genetic and post-harvest factors: A review. *BioMedicine*, 14, 1-8. https://doi.org/10.37796/2211-8039.1451

Sarjadi, M. S., Majid, M. H. A., Laurence, Y. M., & Khan, M. S. (2018). Total flavonoid content and antioxidant activity by different drying and extraction methods of Clinacanthus nutans leaves. *Borneo Science*, 39(2), 72-85. https://doi.org/10.51200/bsj.v39i2.4424

Shang, D., Hu, S., Zhao, Y., Li, Q., & Zhang, J. (2024). Molecular mechanisms of obesity predisposes to atopic dermatitis. *Frontiers in Immunology*, 15, Article 1473105. https://doi.org/10.3389/fimmu.2024.1473105

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Tramontana, M., Dattola, A., Calabrese, G., et al. (2023). Advancing the understanding of allergic contact dermatitis. *Frontiers in Medicine* (Lausanne), 10, Article 1184289. https://doi.org/10.3389/fmed.2023.1184289

US Food and Drug Administration. (2016). Botanical drug development guidance for industry. FDA. https://www.fda.gov/files/drugs/published/Botanical-Drug-Development--Guidance-for-Industry.pdf

World Medical Association. (2013). Declaration of Helsinki: Ethical principles for medical research involving human subjects. *Journal of the American Medical Association*, 310, 2191-2194. https://doi.org/10.1001/jama.2013.281053

Xodo, L. E., Rapozzi, V., Zacchigna, M., Drioli, S., & Zorzet, S. (2012). The chlorophyll catabolite pheophorbide a as a photosensitizer for the photodynamic therapy. *Current Medicinal Chemistry*, 19(6), 799-807. https://doi.org/10.2174/092986712799034879

Yew, Y. W., Thyssen, J. P., & Silverberg, J. I. (2023). Investigating causal relationships between obesity and skin barrier function: A Mendelian randomization analysis. J*ournal of the European Academy of Dermatology and Venereology*, 37(12), 2524-2531. https://doi.org/10.1111/jdv.19326

Zainol, H., & Mansor, H. (2019). A review of therapeutic potentials of Clinacanthus nutans as source for alternative medicines. *Sains Malaysiana*, 48(12), 2683-2691. https://doi.org/10.17576/jsm-2019-4812-09

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