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Anti-Psoriatic Potential of *Rhinacanthus nasutus* in HaCaT Keratinocytes and Imiquimod-Induced Psoriasis Model

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ABSTRACT

Psoriasis is a long-standing inflammatory skin disease that is associated with the uncontrolled increase in the number of keratinocytes and immune system malfunction. The present study evaluated the anti-psoriatic effects of *Rhinacanthus nasutus* (RN), which has a history of use in the field of dermatology, and utilized both *in vitro* and *in vivo* studies. The cytotoxicity of RN solvent extracts (hexane, chloroform, ethyl acetate, and ethanol) was assessed on the HaCaT keratinocytes using the sulforhodamine B (SRB) assay at 12.5 to 200 µg/mL. A significant dose-dependent decrease in HaCaT cell viability was observed, with ethyl acetate (IC_{50}=41.5 µg/mL) and chloroform (IC_{50} = 43.8 µg/mL) extracts being the most potent. The anti-psoriatic effect was studied *in vivo* using an imiquimod (IMQ)-induced psoriasis-like animal model in BALB/c mice, in which RN extracts were administered topically and orally. The psoriasis-like manifestations were significantly improved with treatment as indicated by a lower Psoriasis area severity index (PASI) score, reduced ear thickness, less epidermal hyperplasia, reduced spleen index, and histopathologic changes. There was a significant reduction in post-RN treatment IMQ-induced hematologic disturbances and elevated concentrations of pro-inflammatory cytokines (TNF-α, IL-17A, and IL-23). The ethyl acetate fraction exhibited the strongest therapeutic action compared to all the extracts with a maximum PASI score reduction of 63%, followed by the chloroform extract. Overall, *Rhinacanthus nasutus* has demonstrated a high anti-psoriatic effect by inhibiting hyperproliferation of the keratinocytes and modulating the inflammatory processes.

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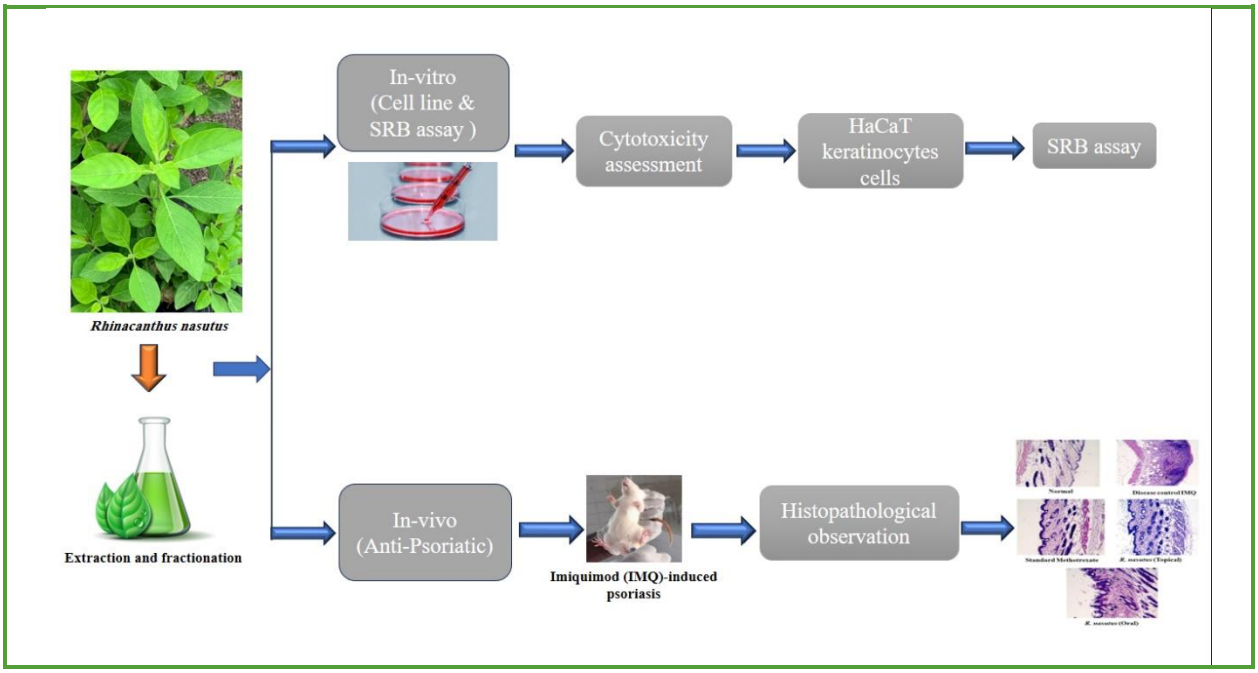
Graphical Abstract



Introduction

The condition of psoriasis is a chronic immune-complex inflammatory skin disorder that presents as scaled and erythematous spots, thus causing significant physical pain and psychosocial distress [1]. The importance of this disorder as one of the significant dermatological conditions is demonstrated by its global prevalence of about 2–3%, indicating that millions of people are affected in both developed and developing countries [2]. The disease burden is substantial in India, with prevalence rates recorded between 0.44 and 2.82%, making it a considerable national public health issue [3].

Psoriasis is not only costly to the management system, but also to patients. It has financial implications due to the financial cost of long-term, and sometimes lifelong, pharmacotherapy, recurrent hospitalizations, and indirect costs in terms of lost productivity and reduced quality of life [4]. This is particularly acute in resource-strained environments, where most patients cannot afford the high costs of modern biologic therapies [5]. Therefore, overcoming the barriers to treatment accessibility, their affordability, and the side effects of traditional agents (*e.g.,* corticosteroids and immunosuppressants, such as hepatotoxicity and nephrotoxicity) is a vital unmet necessity [6]. One potential solution to these challenges lies in seeking safer, multi-targeted, and cost-effective alternatives using medicinal plants [7].

Natural products offer an attractive supply of bioactive compounds, known to possess anti-inflammatory and immunomodulatory properties, which could serve as a potentially viable alternative for managing chronic diseases like psoriasis. One such interesting candidate is a plant known as *Rhinacanthus nasutus (R. nasutus), which* has been utilized in traditional medicine, and according to pre-preliminary studies, it demonstrates strong anti-inflammatory, antioxidant, and antiproliferative effects [8,9]. It has been reported to inhibit some of the major inflammatory pathways mediated

by the cytokines TNF-α, IL-17, and IL-23, which are crucial in the pathogenesis of psoriasis [10].

This gap was identified as the purpose of the present study to test the cytotoxic effects of *R. nasutus* extracts on HaCaT cells to evaluate their ability to regulate the keratinocytes proliferation and to assess the therapeutic effects of *R. nasutus* extracts in an imiquimod-induced psoriasis-like mouse model. The outcome measurements included disease severity (PASI score), epidermal and ear thickness, histopathology, spleen index, hematological parameters, and pro-inflammatory cytokine profiles. Additionally, the extracts (hexane, chloroform, ethyl acetate, and ethanol) were comparatively evaluated based on the solvent used in extraction and the route of administration (topical or oral), to identify the most effective anti-psoriatic extract. This combinative experimental approach provides the mechanistic understanding as well as the empirical evidence supporting the anti-psoriatic effects of "*R. nasutus* extracts, thus establishing them as a more affordable and safer phytotherapeutic alternative for managing psoriasis.

Materials and Methods

Collection and preparation of the extract

Fresh leaves of *R. nasutus* were collected from their natural habitat and authenticated by Dr. K. Jeyaprakash, Tamil Nadu State Plants Board, Chennai (Voucher No. TNSMPB/H/0006). The leaves were shade-dried under controlled conditions and ground into powder. The powdered material was subjected to maceration using hexane, chloroform, ethyl acetate, and ethanol (70%) successively. The extracts were filtered, concentrated under reduced pressure using a rotary evaporator, and vacuum-dried. The final dried extracts were stored at 4 °C until further use [11,12].

Experimental conditions

Cytotoxicity in relation to psoriasis was assessed with a tested i*n vitro* system constituting the human keratinocyte cell line HaCaT. Cells were kept in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin solution. The cultures grew at 37 °C under a moist environment with the addition of 5% CO_2. When the confluency reached about 80%, the cells were detached with 0.25% trypsin–EDTA. In the cytotoxicity experiment, HaCaT cells were cultured in 96-well plates at a density of 1×10^4 cells per well and allowed to adhere for 24 h before treatment [13-15].

Cytotoxicity assay (SRB method)

The sulforhodamine B (SRB) assay was used to determine the cytotoxic potential of the plant extracts. HaCaT cells were incubated with the various concentrations of each extract (25, 50, 100, and 200 µg/mL) and left to incubate for 48 h. After treatment, cells were fixed using 10% trichloroacetic acid (TCA) and stained using 0.4% SRB solution in 1% acetic acid. Unbound dye was washed repeatedly with 1% acetic acid to eliminate excess dye, and the protein-bound dye was solubilized with 10 mM Tris base (pH 10.5). A microplate reader was used to measure absorbance at 570 nm. Cell viability was expressed as a percentage relative to untreated control cells. Methotrexate served as the reference standard. IC_{50} values were calculated from dose–response curves using curve-fitting software [16-18].

Animals and ethical considerations

Healthy Swiss albino mice of either sex, weighing 20–30 g, were used for the study.

The animals were obtained from Radiant Research (Reg. No. 1803/PO/RcBi/S/2015/CPCSEA; dated 08/09/2022). Animals were housed under standard laboratory conditions with a 12 h light/dark cycle, controlled temperature (25 ± 2 °C), and relative humidity (55 ± 5%). They were provided free access to a standard pellet diet and water ad libitum (Table 1). All experimental procedures were conducted in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Grouping and treatment

Acute dermal toxicity (OECD 402)

Acute dermal toxicity testing was conducted in compliance with OECD Guideline 402 (OECD, 2017). A preliminary dose-finding study was performed with 1000 mg/kg extract applied topically on clipped dorsal skin, followed by gauze dressing to prevent ingestion. After 24 h exposure, residual extract was removed, and animals were monitored up to 14 days for clinical signs, mortality, and local skin reactions. In the main study, extracts were applied at the highest non-lethal dose to additional groups. Parameters observed included changes in body weight, clinical signs, gross pathology, and skin reactions (erythema, edema). Irritation scoring followed the Draize method, and the primary irritation index (PII) was calculated. LD_{50} determination and classification were carried out according to the Globally Harmonized System (GHS) [19,20].

Hematological and biochemical analysis

At the end of the study, blood samples were collected via retro-orbital plexus under light anesthesia. Hematological parameters such as neutrophils, lymphocytes, eosinophils, monocytes, RBC, Hb, platelets, PCV, MCV, MCH, and MCHC were analyzed using an automated hematology analyzer [21].

Skin condition assessment

Psoriatic severity was scored using the Psoriasis Area and Severity Index (PASI). Ear thickness and epidermal thickness were measured using vernier calipers and histological sections, respectively. Pro-inflammatory cytokines (TNF-α, IL-17A, and IL-23) were quantified in skin tissue homogenates by ELISA kits. Histopathological scoring was carried out on H&E-stained sections based on epidermal hyperplasia, parakeratosis, and inflammatory infiltrates (scale 0–4) [22-25].

Table 1. Experimental animal grouping and treatment protocol

Sr./ No.	Group	Treatment	Number of mice
1	Normal control	Vehicle only	3
2	Disease control (IMQ)	Imiquimod (5% cream, 62.5 mg/day, ear and dorsal skin)	3
3	Standard control	Methotrexate (1 mg/kg)	3
4	RNS1 (Ethyl acetate extract) – Oral	Extract (dose as per study)	3
5	RNS1 (Ethyl acetate extract)– Topical	Extract formulation	3
6	RNS2 (Chloroform extract) – Oral	Extract (dose as per study)	3
7	RNS2 (Chloroform extract) – Topical	Extract formulation	3

Results and Discussion

Cytotoxicity (HaCaT cell viability)

The percentage viability of HaCaT cells at 12.5–200 µg/mL is presented in Table 2. IC_{50} analysis (Table 3) confirmed this trend: ethyl acetate (41.5 µg/mL) and chloroform (43.8 µg/mL) extracts showed moderate to high cytotoxicity, whereas hexane (55.0 µg/mL) and ethanol (60.0 µg/mL) were less potent. MTX (25.7 µg/mL) remained the most effective, as illustrated in Figure 1. Overall, the semi-polar fractions (ethyl acetate and chloroform) were more active than the polar or non-polar extracts, suggesting solvent polarity influences the extraction of bioactive compounds. The effect of *R. nasutus* extracts on body weight and spleen indices is presented in Table 4. Both RNS1 (ethyl acetate) and RNS2 (chloroform) treatments caused slight increases in body weight (32.59 ± 0.37 g and 32.65 ± 0.25 g, respectively) compared with the control group (31.97 ± 0.61 g). Notably, spleen weights were significantly reduced in treated groups, with RNS1 at 0.13 ± 0.01 g and RNS2 at 0.12 ± 0.01 g versus 0.21 ± 0.02 g in controls. Correspondingly, spleen indices decreased from 16.65 ± 0.89 in controls to 12.00 ± 0.56 (RNS1) and 10.6 ± 1.3 (RNS2). These findings indicate that both extracts effectively modulated splenic hypertrophy, suggesting potential immunomodulatory effects.

Table 2. Viability of HaCaT cells treated with *R. nasutus* extracts

Sr./ No.	Concentration (µg/mL)	Ethyl acetate extract (%)	Chloroform extract (%)	Hexane extract (%)	Ethanol extract (%)	Methotrexate (STD) (%)
1	0.0	100.0	100.0	100.0	100.0	100.0
2	12.5	78.6	85.0	83.1	86.7	62.7
3	25.0	66.8	78.4	76.5	80.0	48.6
4	50.0	54.3	67.9	69.2	69.5	33.5
5	100.0	40.1	51.3	63.0	57.6	17.1
6	200.0	27.3	35.1	41.8	41.1	7.9

Table 3. IC_{50} Values of plant extracts compared to methotrexate

Sr./No.	Extract & Solvent	IC_{50} (µg/mL)	Cytotoxicity profile
1	*R. nasutus* – Ethyl Acetate	41.5	Moderate–High
2	*R. nasutus* – Chloroform	43.8	Moderate–High
3	*R. nasutus* – Hexane	55.0	Moderate
4	*R. nasutus* – Ethanol	60.0	Moderate–Low
5	Methotrexate (Standard)	25.7	High

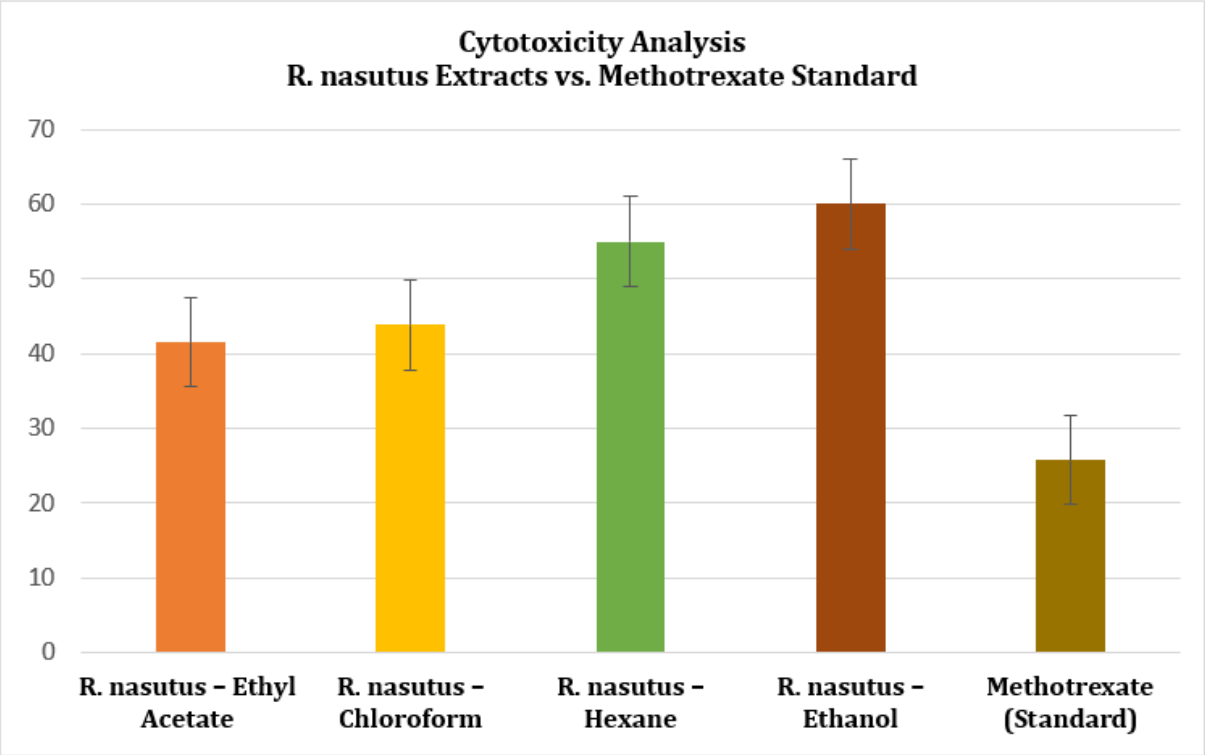


Figure 1. IC_{50} values of plant extracts vs methotrexate

Table 4. Weight parameters

Sr./No.	Group	Body weight (g)	Spleen weight (g)	Spleen index
1	Control	31.97 ± 0.61	0.21 ± 0.02	16.65 ± 0.89
2	RNS1 (Ethyl acetate)	32.59 ± 0.37	0.13 ± 0.01	12.00 ± 0.56
3	RNS2 (Chloroform extract)	32.65 ± 0.25	0.12 ± 0.01	± 1.3

Hematological parameters

IMQ administration in diseased mice caused significant hematological disturbances, including reduced neutrophils (7.26%), elevated lymphocytes (91.6%), eosinophils (7.3%), monocytes (7.0%), decreased hemoglobin (7.89 g/dL), RBCs (2.11 million/cmm), platelets (1.65 L/cmm), and abnormal red cell indices, indicating systemic inflammation and anemia. Treatment with methotrexate restored leukocyte and erythrocytic parameters close to normal. Both topical and oral *R. nasutus* extracts (RNS1 and RNS2) significantly corrected these imbalances. Topical RNS1/RNS2 reduced lymphocytes to 85.6 and 81.8%, while oral RNS1/RNS2 normalized them to 75.2 and 71.0%. Eosinophils, monocytes, RBCs, hemoglobin, platelets, and red cell indices improved, with oral RNS1 (ethyl acetate) showing the best effects, comparable to the standard drug. These results indicate that *R. nasutus* extracts, especially oral ethyl acetate extract (RNS1), protect against IMQ-induced leukocytosis and anemia, demonstrating potent systemic immunomodulatory and hematological stabilizing effects.

Table 5. Effect of *R. nasutus* on disease severity and inflammatory indices in IMQ-induced psoriasis model

Sr./No.	Parameter	Normal control	IMQ control	Standard methotrexate	*R. nasutus* (topical)	*R. nasutus* (oral)
1	PASI Score (0–12)	0.5 ± 0.1	10.2 ± 0.3	2.3 ± 0.2***	3.8 ± 0.3**	4.2 ± 0.4**
2	Ear Thickness (mm)	0.23 ± 0.02	0.68 ± 0.03	0.28 ± 0.01***	0.34 ± 0.02**	0.36 ± 0.03**
3	Epidermal thickness (μm)	24.6 ± 1.4	128.3 ± 3.5	38.1 ± 1.6***	52.6 ± 2.1*	59.4 ± 2.7**
4	TNF-α (pg/mg)	18.5 ± 1.2	84.2 ± 2.5	26.8 ± 1.5***	39.3 ± 2.0*	42.5 ± 1.8*
5	IL-17A (pg/mg)	10.3 ± 0.9	76.5 ± 3.0	21.1 ± 1.2***	32.7 ± 1.9**	35.0 ± 2.1**
6	IL-23 (pg/mg)	14.7 ± 1.0	91.6 ± 2.8	24.5 ± 1.3***	36.8 ± 1.6*	39.2 ± 2.0**
7	Spleen weight (g)	0.095 ± 0.01	0.21 ± 0.02	0.11 ± 0.01**	0.13 ± 0.01*	0.14 ± 0.01*
8	Histopath score (0–4)	0.3 ± 0.1	3.8 ± 0.2	1.0 ± 0.1***	1.5 ± 0.2**	1.7 ± 0.2**

Values are expressed as mean ± SEM (n = 6).
*Statistical analysis was performed using one-way ANOVA followed by appropriate post-hoc test. *p < 0.05, **p < 0.01, ***p < 0.001 compared with IMQ control group*

Skin condition improvement

Effect of *R. nasutus* extracts on PASI score, ear thickness, epidermal thickness, cytokines, spleen weight, and histopathological score in IMQ-induced psoriatic mice. Representative photomicrographs of H&E-stained skin sections are shown in Figure 2.

IMQ treatment induced a significant psoriatic phenotype in mice, as evidenced by elevated PASI scores, ear and epidermal thickness, pro-inflammatory cytokines (TNF-α, IL-17A, and IL-23), spleen weight, and histopathological scores compared to normal controls ($p < 0.001$; Table 5 and Figure 3). Methotrexate treatment significantly attenuated these alterations (***$p < 0.001$ vs. IMQ). *R. nasutus* extracts administered topically and orally also improved all parameters, with significant reductions in PASI score, ear and epidermal thickness, and cytokine levels compared to the IMQ group ($p < 0.05$ or $p < 0.01$). The disease control group (A) showed typical psoriatic features, including severe hyperkeratosis, acanthosis, elongated rete ridges, and dense inflammatory infiltration. Oral administration of *R. nasutus* (B) significantly reduced epidermal thickening, hyperkeratosis, and inflammatory infiltration. Marked local improvement was observed with topical treatment (C), characterized by mild hyperplasia, reduced hyperkeratosis, and moderate inflammation. Methotrexate-treated skin (D) exhibited near-normal architecture with minimal hyperplasia and low inflammatory cell infiltration, indicating high therapeutic efficacy.

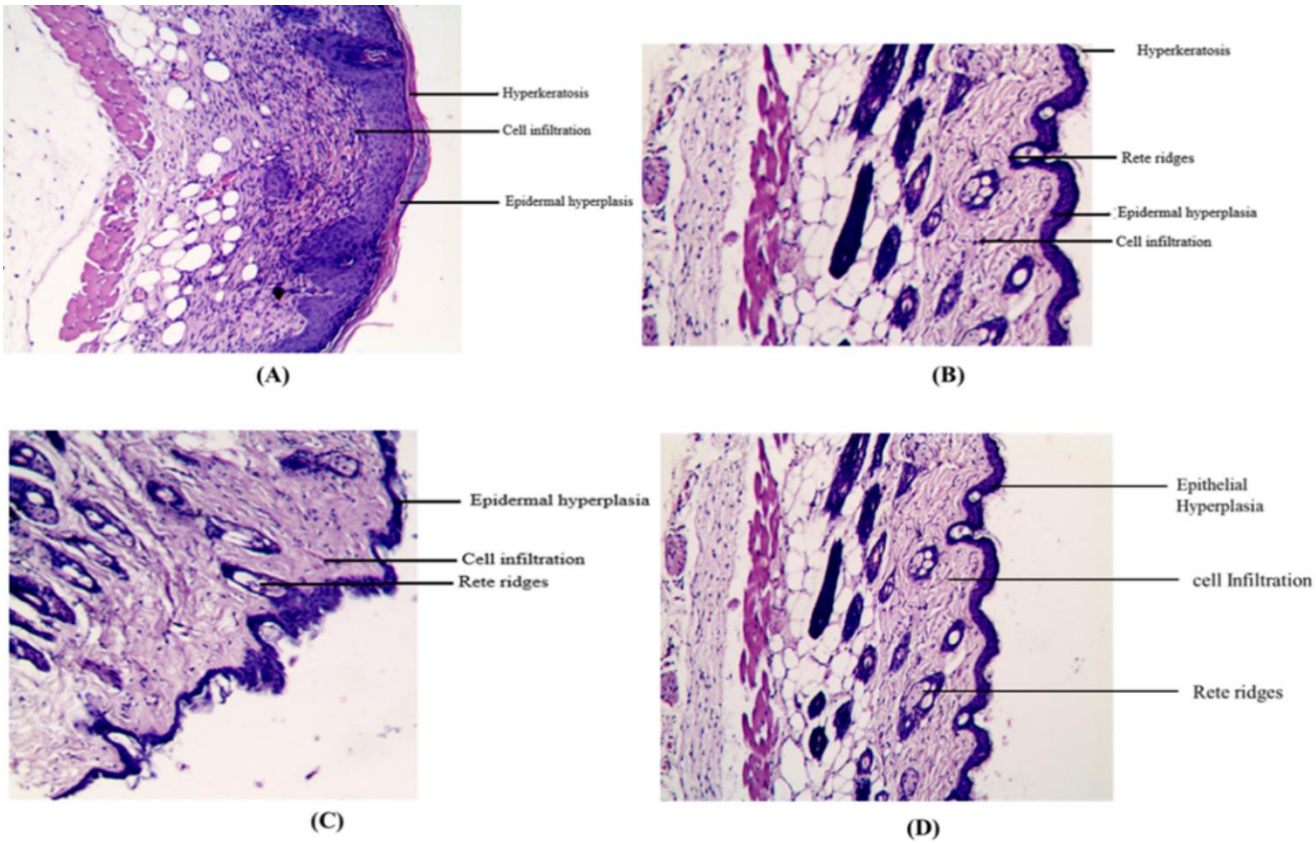


Figure 2. Histopathological evaluation of skin sections from IMQ-induced psoriatic mice treated with *R. nasutus* extract and standard drug

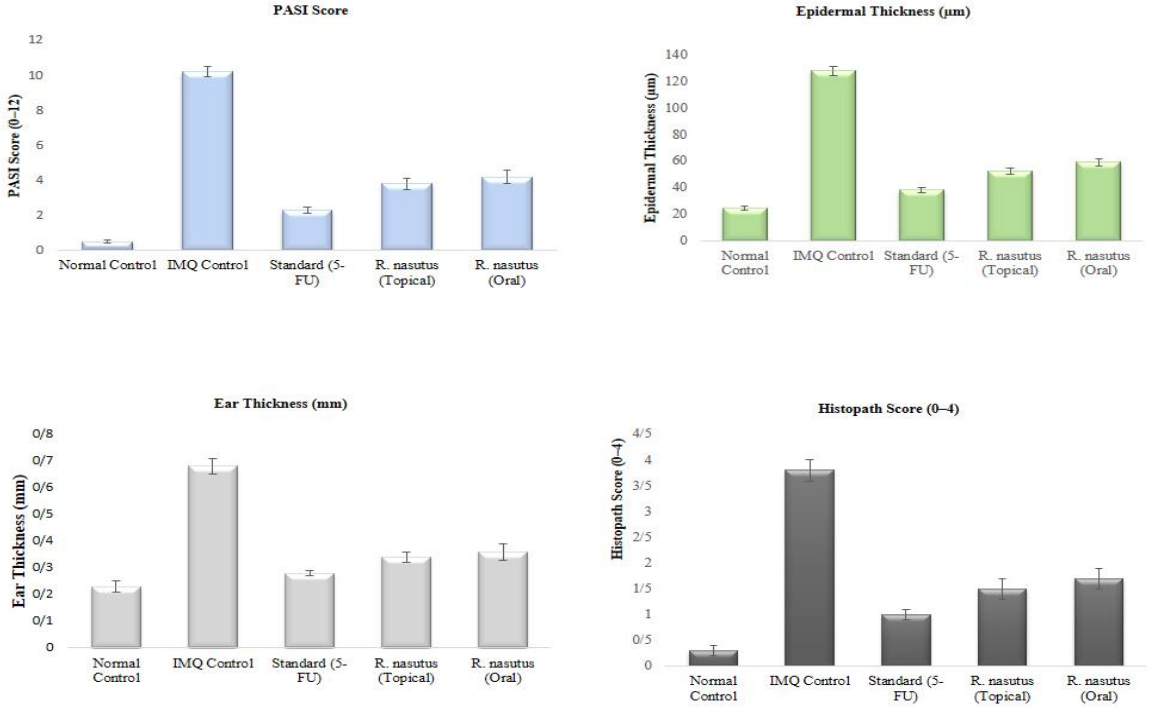


Figure 3. Illustration of PASI score (a), epidermal thickness (b), ear thickness (c), and histopathological score (d) in IMQ-induced psoriatic mice

Conclusion

The current research has shown that *R. nasutus* extracts have potent anti-psoriatic–like effects by both inhibiting the hyperproliferation of keratinocytes and suppressing the production of inflammatory mediators. The semi-polar fractions (ethyl acetate, RNS1, IC_{50}: 41.5 µg/mL and chloroform, RNS2, IC_{50}: 43.8 µg/mL) displayed high levels of cytotoxicity against HaCaT keratinocytes i*n vitro*, suggesting their involvement in regulating abnormal epidermal growth. Topical and oral administration of ethyl acetate and chloroform extracts *in vivo* significantly decreased PASI scores, epidermal and ear thickness, pro-inflammatory cytokines (TNF-α, IL-17A, and IL-23), spleen hypertrophy, and overall disease severity in IMQ-induced psoriatic mice. Interestingly, topical application of the ethyl acetate extract (RNS1) produced the highest efficacy, with a 63% improvement in PASI scores, followed by topical application of the chloroform extract, both of which restored skin architecture and alleviated inflammation more effectively compared to oral administration. These findings indicate that *R. nasutus* and especially its ethyl acetate and chloroform fractions (RNS1 and RNS2), can be a promising phytotherapeutic agent in the management of psoriasis as they inhibit the development of the skin and its immune mechanisms to regulate the pathogenesis. *In silico* analysis further corroborated the findings, identifying rhinacanthin D from *R. nasutus* as a stable, drug-like multi-target anti-psoriatic lead with strong binding affinity toward key psoriasis targets (PDE4D, TYK2, and S1P1R).

Abbreviations

ANOVA: Analysis of variance; BALB/c: Inbred strain of laboratory mice; CO_2: Carbon dioxide; CPCSEA: Committee for the purpose of control and supervision of experiments on animals; DMEM: Dulbecco's modified eagle medium; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme-linked immunosorbent assay; FBS: Fetal Bovine serum; GHS: Globally harmonized system; Hb: Hemoglobin; HaCaT: Human adult low-calcium high-temperature keratinocyte cell line; H&E: Hematoxylin and eosin; IC_{50}: Half-maximal inhibitory concentration; IL: Interleukin; IMQ: Imiquimod; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; MTX: Methotrexate; OECD: Organisation for economic co-operation and development; PASI: Psoriasis area and severity index; PCV: Packed cell volume; PDE4D: Phosphodiesterase 4D; PII: Primary irritation index; RBC: Red blood cells; RN: *R. nasutus*; RNS1: *R. nasutus* ethyl acetate extract; RNS2: *R. nasutus* chloroform extract; SEM: Standard error of the mean; S1P1R: Sphingosine-1-phosphate receptor 1; SRB: Sulforhodamine B; STD: Standard drug; TNF-α; Tumor necrosis factor-alpha; and TYK2: Tyrosine kinase 2.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this manuscript.

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