Annals of Phytomedicine 15(1): 720-726, 2026

DOI: http://dx.doi.org/10.54085/ap.2026.15.1.64

Annals of Phytomedicine: An International Journal
http://www.ukaazpublications.com/publications/index.php

Print ISSN : 2278-9839 Online ISSN : 2393-9885

Original Article : Open Access

Evaluation of the antifungal efficacy of *Acanthus ilicifolius* L. extract ointment in canine mycotic dermatitis

A.S. Khairmode*, V.V. Karande*◆, B.C. Ghumare*, U.M. Tumlam, B.N. Ambore***, S.N. Jadhav**** and M. B. Bukke***

* *Department of Veterinary Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Sciences (Affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur), Shirwal, Satara-412801, Maharashtra, India*

** *Department of Veterinary Microbiology, Krantisinh Nana Patil College of Veterinary Sciences (Affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur), Shirwal, Satara-412801, Maharashtra, India*

*** *Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Krantisinh Nana Patil College of Veterinary Sciences (Affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur), Shirwal, Satara-412801, Maharashtra, India*

**** *Department of Veterinary Biochemistry, Krantisinh Nana Patil College of Veterinary Sciences (Affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur), Shirwal, Satara-412801, Maharashtra, India*

Article Info

Article history
Received 19 April 2026
Revised 29 May 2026
Accepted 30 May 2026
Published Online 30 June 2026

Keywords
Acanthus ilicifolius L.
Canine
Mycotic dermatitis
Antifungal ointment
Dermatology

Abstract

Canine mycotic dermatitis remains a longstanding challenge in veterinary practice and might resulted due to growing resistance of current antifungals and adverse effects associated with them. A mangrove-associated medicinal plant, *Acanthus ilicifolius* L. (Family: Acanthaceae), and documented anti-inflammatory and antifungal properties with its bioactive phytoconstituents including flavonoids, triterpenoids, alkaloids, and phenolic acids. The study was designed to evaluate the antifungal potential and systemic safety of a prepared topical ointment using methanolic extract of *A. ilicifolius* leaves in naturally occurring canine mycotic dermatitis. Confirmed diagnosed 30 cases were recruited in the study and divided in ten animals in each. Group I received 2% topical ketoconazole; Group II received 5% w/w formulation; and Group III received 10% w/w formulation of extract in ointment form. Clinical, mycological, haematological, and biochemical parameters were assessed at Day 0 and Day 28 of the study. Extraction yield was found to be 18.8%. Both formulations exhibited acceptable physicochemical properties (pH 6.2-6.4). Principal fungal isolates from all the subjects were *Aspergillus* spp. (26.67%), *Microsporum canis* (23.33%), *Candida albicans* (20.00%), *Malassezia pachydermatis* (16.67%), and *Trichophyton mentagrophytes* (13.33%). *In vitro* antifungal activity on agar well was concentration-dependent. Clinically, a complete cure was achieved in 66.7% of cases and marked improvement in the remaining 33.3%, with no treatment failures. Group III (10% extract) attained a 60% recovery rate, approaching Group I (70%), and significantly surpassing Group II (50%). Significant reductions in total leucocyte counts and eosinophil percentages confirmed resolution of systemic fungal inflammation. Serum alkaline phosphatase declined significantly across all treated groups, with F1 showing the greatest absolute reduction, suggesting hepatoprotective activity. No adverse reactions or pathological haemato-biochemical changes were recorded, confirming a safety profile as per ketoconazole. These findings establish *A. ilicifolius* extract ointment as a promising, cost-effective phytotherapeutic alternative for canine mycotic dermatitis.

1. Introduction

Fungal infections of skin constitute a considerable portion of dermatological disorders in small animal veterinary practice, with mycotic dermatitis accounting for an estimated 15-30% of canine dermatological consultations (Hill *et al.,* 2006; Miller *et al.,* 2013). In India, warm, humid climatic conditions, particularly prevalent in Maharashtra's tropical belt, create optimal conditions for fungal proliferation, and recent epidemiological data indicate that approximately 4.77% of dogs presented to veterinary clinics were diagnosed with fungal dermatitis (Patel *et al.,* 2024). The principal causative organisms include dermatophytes, notably *M. canis*, *T. mentagrophytes*, and *M. gypseum* and opportunistic yeasts such as *M. pachydermatis* and *Candida* species, each capable of inducing the hallmark clinical features such as pruritus, alopecia, erythema, and scaling (Moriello *et al.,* 2017; Guillot and Bond, 2020).

Conventional antifungal pharmacotherapy relies on azoles (ketoconazole, itraconazole, fluconazole), allylamines, and polyenes. Azole antifungals act by suppressing the cytochrome P450-dependent enzyme lanosterol 14 α-demethylase, which resulted in disruption of ergosterol biosynthesis and breaks the integrity of fungal membrane (Ghannoum and Rice, 1999). However, escalating global rise of resistance to antifungal drug, hepatotoxic adverse effects associated with systemic azoles, and the relatively limited antifungal arsenal available in veterinary medicine have prompted urgent investigation of alternative therapeutic strategies (Cowen *et al.,* 2014; Perlin *et al.,* 2017; Fisher *et al.,* 2018). Resistance ranging from 5 to 20% among clinical isolates, depending on geographic region and prior antifungal exposure, has been documented for ketoconazole in veterinary fungal isolates (Cafarchia *et al.,* 2012; Sharma *et al.,* 2021).

Corresponding author: Dr. V. V. Karande
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Krantisinh Nana Patil College of Veterinary Science (Affiliated to Maharashtra Animal and Fishery Sciences University, Nagpur), Shirwal, Satara-412801, Maharashtra, India
E-mail: vikaskarande@mafsu.ac.in
Tel.: +91-9029802323

Plant-derived antifungals have attracted considerable attention as complementary or alternative treatments owing to their chemical diversity, multitarget mechanisms of action, reduced propensity for resistance selection, and accessibility in resource-limited settings (Abad *et al.,* 2007; Newman and Cragg, 2016; Villar *et al.,* 2023). *A. ilicifolius* (Family: Acanthaceae), commonly known as sea holly or Marandi in Maharashtra, is a mangrove-associated medicinal plant found all over tropical and subtropical coastal regions of Asia, Africa, as well as Australia. In traditional systems of medicine, comprising of Ayurveda, Unani, and traditional medicine of China, it has been employed to treat skin diseases, rheumatism, asthma, and infectious disorders (Babu *et al.,* 2012; Singh and Aeri, 2013; Tripathi *et al.,* 2024). Phytochemical investigations have identified flavonoids (apigenin, luteolin, quercetin), triterpenoids (lupeol, â-sitosterol, stigmasterol), alkaloids, tannins, saponins, and phenolic acids as the principal bioactive constituents with established antifungal, anti-inflammatory, antioxidant, and immunomodulatory properties (Babu *et al.,* 2012; Pothiraj *et al.,* 2021; Matos *et al.,* 2022).

Prior *in vitro* studies have demonstrated significant inhibitory activity of *A. ilicifolius* leaf extracts against dermatologically relevant fungi, including *T. rubrum*, *M. canis*, *Candida albicans*, and *Aspergillus* species (Khajure and Rathod, 2010; Kalaskar *et al.,* 2012; Govindasamy and Arulpriya, 2013). Despite these promising preliminary findings, no controlled clinical study has evaluated *A. ilicifolius* extract in a topical ointment formulation against naturally occurring canine mycotic dermatitis. In view of all this, the current investigation was planned to develop and characterise topical ointment formulations containing 5% and 10% methanolic extract of *A. ilicifolius;* and to isolate and identify the principal fungal agents associated with canine mycotic dermatitis in the study population; as well as to evaluate the antifungal efficacy, anti-inflammatory activity, and systemic safety of these formulations relative to standard ketoconazole therapy.

2. Materials and Methods

2.1 Plant identification

Whole plant, along with leaves and root, was sent to a botanist for identification at Department of Botany, Yashwantrao Chavan Institute of Science, Satara, Maharashtra, India. The plant was identified and provided Accession Number as KAS001.

2.2 Extract preparation

A. ilicifolius fresh leaves were collected from the Konkan coastal region of Maharashtra during the months of November and December 2025. After getting confirmation from a botanist, the collected plant material was washed carefully, the leaves were shade-dried, and pulverised to make it a powder form. Continuous extraction was performed with methanol as the solvent at 45-50°C using Soxhlet's apparatus, until the recycled solvent appeared colourless. The resultant semiliquid extract was subjected to filtration using Whatman No. 1 filter paper, and further solvent evaporation was done at 50°C to make the extract concentrated, and preserved in airtight glass containers at 4°C. Extractability percentage was obtained using the following formula: Extractability (% w/w) = [Final Weight of dried extract (g) /Initial Weight of leaf powder (g)] × 100

2.3 Phytochemical analysis

Preliminary qualitative phytochemical investigations were conducted using leaf extract of *A. ilicifolius* to find out the presence of various phytochemicals, comprising carbohydrates, alkaloids, glycosides, flavonoids, phytosterols, saponins, proteins, and tannins, following standard protocols for each class of phytochemical. For each class of phytochemical, as per the methods described in earlier literature.

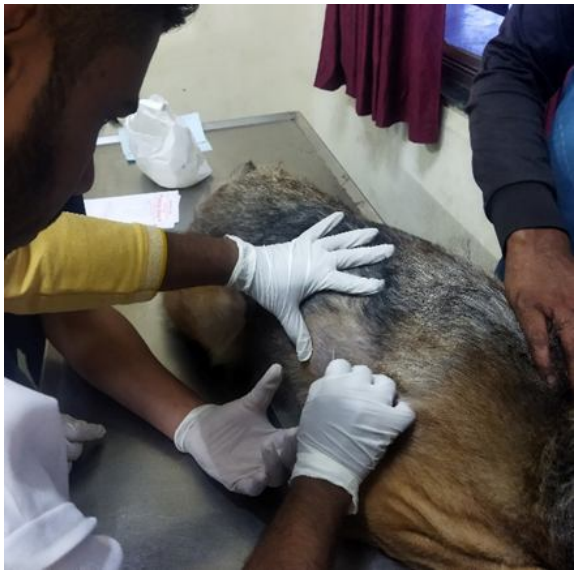

Figure 1: Collection of skin scraping from the affected lesion for mycological examination.

Figure 2: Prepared methanolic extract of *A. ilicifolius*.

2.4 Preparation of ointment

Fusion method was used to prepare a simple oleaginous ointment base, comprising hard paraffin (10% w/w), white soft paraffin (85% w/w), and wool fat/lanolin (5% w/w), melted sequentially in a water bath at 60-65°C with continuous stirring. Two medicated formulations were prepared: Formulation F1 (5% w/w *A. ilicifolius* extract + 2% w/w salicylic acid) and Formulation F2 (10% w/w extract + 2% w/w salicylic acid). The extract was dissolved in dimethyl sulphoxide (DMSO) to facilitate uniform dispersion. Salicylic acid and dissolved

extract were incorporated into the cooled base (40-45°C) by geometric dilution with continuous stirring during gradual cooling to maintain uniform consistency. The formulations were transferred to clean, dry, labelled containers and stored at 25 ± 2°C. Both formulations were evaluated for physical appearance, homogeneity, and pH.

2.5 Experimental animals and study design

The study was conducted at the veterinary clinical complex (VCC), Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara, Maharashtra, following institutional ethical guidelines as per the CCSEA norms. Thirty dogs with clinical signs suggestive of mycotic dermatitis, multifocal circular alopecic patches, pruritus, erythema, and scaling, and confirmed by direct microscopy (KOH preparation) and fungal culture, were enrolled in the study. Animals were arbitrarily separated into three equal groups (n = 10): Group I (standard control) received 2% ketoconazole topical preparation; Group II received F1 (5% extract ointment); and Group III received F2 (10% extract ointment). All formulations were applied topically twice daily for 28 days. Clinical, mycological, haematological, and biochemical parameters were recorded at Day 0 (pre-treatment) and Day 28 (post-treatment).

2.6 Clinical diagnosis

Skin scraping, along with hair samples were collected from the active margins of lesions under aseptic conditions. Microscopic examination employed a 10-20% solution of potassium hydroxide (KOH) to demonstrate fungal elements. Samples were cultured on sabouraud dextrose agar (SDA), incubated at 25-28°C for up to 21 days, and examined every 2-3 days. Colony identification was based on macroscopic morphology (texture, colour, and pigmentation) and confirmed microscopically using lactophenol cotton blue (LPCB) staining at 10x and 40x magnification.

2.7 *In vitro* antifungal activity

Antifungal potential of F1 and F2 was examined against the five clinically isolated fungal species using the method of agar well diffusion following standardised procedures (Balouiri *et al.,* 2016; CLSI, 2017). SDA plates were uniformly inoculated with standardised fungal suspensions; well punching of 6mm diameter was done aseptically, and test ointments including F1, F2, and ketoconazole 2% were deposited into the wells. Further plates were subjected to incubation at 25-28°C for 5-7 days, and zones of inhibition were measured in duplicate with the help of a Vernier calliper. The ointment application procedure and supportive treatment schedule.

2.8 Haematological and biochemical parameters

Blood was collected from the cephalic vein at Day 0 and Day 28. Blood Samples were analysed for haemoglobin (Hb), packed cell volume/haematocrit (PCV), red blood cell count (RBC), platelet count, total leucocyte count (TLC), and differential leucocyte count (DLC). Serum was obtained by centrifugation (3000 rpm, 10 min) and analysed using a semi-automatic biochemistry analyser for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, and globulin, total protein (TP). Statistical comparisons within and between groups were performed by independent sample t-tests; $p<0.05$ was considered significant.

3. Results

3.1 Extraction and formulation characteristics

Soxhlet methanolic extraction of 1000 g of *A. ilicifolius* leaf powder yielded 188 g of concentrated dark greenish-brown, semi-solid extract, corresponding to an extract yield of 18.8% w/w. This yield was consistent with previously reported values for *A. ilicifolius* and indicates a good extraction yield of polar and semi-polar phytoconstituents (Pothiraj *et al.,* 2021; Natarajan *et al.,* 2024). Both ointment formulations exhibited smooth, homogeneous consistency without phase separation or grittiness. The pH of F1 (6.2 ± 0.15) and F2 (6.4 ± 0.18), which was within the accepted range for canine skin (6.2-7.5), ensures compatibility, maintenance of the skin surface environment, and may support antifungal activity of salicylic acid in the acidic microenvironment.

3.2 Phytochemical analysis

Phytochemical analysis of the extract of *A. ilicifolius* shows the presence of sugar, phenols, saponins, proteins, tannins, resins and glycosides. The earlier research report of Govindsamy and Arulpriya (2013) supports the present finding.

3.3 Epidemiological profile of canine mycotic dermatitis

Mycotic dermatitis was mostly observed in dogs aged 5-10 years (37%), followed by the dogs upto 5 year age group (30%), with the combined 1-10 year cohort representing 67% of cases. A mild male predisposition was noted (male: female = 1.3:1; 57% male).

3.4 Clinical presentation

All 30 dogs exhibited pruritus (100%); with additional clinical signs including alopecia (93.3%), erythema (90%), scaling/crusting (83.3%), self-excoriation (73.3%), hyperpigmentation (60%), and secondary bacterial infection (40%). Lesions showed anatomical predilection for the pinnae and ear canal (80%), face and muzzle (66.7%), ventral abdomen (63.3%), paws and interdigital spaces (56.7%), and axillae/groin (53.3%).

3.5 Mycological findings

Fungal growth was obtained from 28 of 30 samples (93.3%), with the majority of isolates (53.33%) showing visible growth within 6-10 days of incubation on SDA. Staining with lactophenol cotton blue continued species-level identification under the microscope. Five fungal taxa was identified (Table 1): *Aspergillus* spp. were the most frequent isolates (26.67%), followed by *M. canis* (23.33%), *C. albicans* (20.00%), *M. pachydermatis* (16.67%), and *T. mentagrophytes* (13.33%).

3.6 *In vitro* antifungal activity

A. ilicifolius ointment formulations exhibited significant, concentration-dependent antifungal activity against all five fungal species tested (Table 2). Ointment with 10% concentration (F2) demonstrated inhibition zones of 15.8-21.3 mm across the five pathogens, achieving 78.2-82.9% of the efficacy of 2% ketoconazole, while F1 (5%) produced zones ranging between 10.2-15.1 mm (approximately 49.7-58.8% of ketoconazole activity). *M. pachydermatis* showed the highest susceptibility to both formulations (F2: 21.3 mm), attributable to the lipophilic properties of *A. ilicifolius* phytoconstituents facilitating interaction with the lipid-enriched yeast cell membrane.

Table 1: Distribution and macroscopic/microscopic identification of fungal isolates in canine mycotic dermatitis (n = 30)

Fungal species	Isolates (n)	Frequency (%)	Key features
Aspergillus spp.	8	26.67	Septate hyphae, conidiophores with vesicles, radiate conidia
M. canis	7	23.33	Fusiform thick-walled macroconidia (6-15 septa), golden reverse
C. albicans	6	20.00	Oval budding yeasts, pseudohyphae, germ tube positive
M. pachydermatis	5	16.67	Bottle-shaped yeasts, monopolar budding, 'footprint' appearance
T. mentagrophytes	4	13.33	Spherical microconidia in grape-like clusters, spiral hyphae

Table 2: Mean zones of inhibition (mm) of *A. ilicifolius* ointment formulations and ketoconazole control against fungal isolates (agar well diffusion assay)

Fungal species	Ketoconazole 2%(in mm)	F1 (5% extract) (in mm)	F2 (10% extract) (in mm)
Aspergillus spp. (n=8)	20.2	10.2	15.8
M. canis (n=7)	23.8	13.2	19.4
C. albicans (n=6)	24.2	14.0	19.5
M. pachydermatis (n=5)	25.7	15.1	21.3
T. mentagrophytes (n=4)	21.5	11.8	17.6

3.7 Therapeutic outcomes

Clinical recovery outcomes of all groups are summarised in Table 3. Group I (ketoconazole) achieved the highest recovery rate (70%; 7/10 dogs) with complete resolution of pruritus, scales, crusts, and erythema by Day 28 of the study. Group III (10% *A. ilicifolius*) attained a recovery rate of 60% (6/10 dogs) with marked improvement in all principal clinical parameters; Group II (5%) achieved 50% recovery, which was the lowest among all. Combining all groups, 66.7% of cases attained complete clinical cure and 33.3% demonstrated marked improvement, with no treatment failures recorded in any group.

3.8 Haematological parameters

Haematological parameters were assessed on day 0 and 28 of the study, and the same is represented in Table 4. Haemoglobin, haematocrit, RBC count, and platelet count remained within the normal physiological range in all groups throughout the study period, confirming the absence of adverse haematological changes attributable to topical ointment application (Table 4). Total leucocyte count (TLC), elevated above the normal reference range from baseline in Groups I and II, reflecting the leucocytosis associated with active fungal infection and inflammation (Nafie *et al.,* 2021), significantly by Day 28 in Group I (17.08 ± 1.48 to 12.09 ± 0.59 × 10^3/µl) and similarly in Group II (19.02 ± 2.11 to 13.39 ± 0.51 × 10^3/µl), indicating effective resolution of systemic inflammation. Eosinophil percentages declined significantly across all treatment groups as follows: Group I: 5.90 ± 0.74 to 1.40 ± 0.34%; Group II: 3.80 ± 0.63 to 1.20 ± 0.25%; Group III: 4.90 ± 0.62 to 0.50 ± 0.26%, reflecting attenuation of the T-helper 2 cell-mediated hypersensitivity response characteristically associated with mycotic skin disease (Chen *et al.,* 2002; Nafie *et al.,* 2021)

Table 3: Comparative therapeutic recovery in dogs with mycotic dermatitis (n = 30)

Group	Treatment	n	Recovered (n)	Recovery rate (%)
I	Ketoconazole 2% topical	10	7	70.0
II (F1)	*A. ilicifolius* 5% ointment	10	5	50.0
III (F2)	*A. ilicifolius* 10% ointment	10	6	60.0.

Table 4: Key haematological parameters (mean ± SE) at Day 0 and Day 28 across treatment groups

Parameterdays	Group I (ketoconazole)		Group II (F1, 5%)		Group III (F2, 10%)	
	0	28	0	28	0	28
Hb (g/dl)	13.31 ± 0.73	14.27 ± 0.42	14.48 ±0.64	14.77 ± 0.37	13.59 ± 0.68	14.14 ± 0.24
TLC (×10^3/µl)	17.08 ± 1.48	12.09 ±0.59*	19.02 ±2.11	13.39 ± 0.51*	16.02 ± 3.35	13.41 ± 2.29
Eosinophils (%)	5.90 ± 0.74	1.40 ± 0.34***	3.80 ± 0.63	1.20±0.25***	4.90 ± 0.62	0.50 ± 0.26***

Hb = Haemoglobin; TLC = Total leucocyte count. *p<0.05; **p<0.01; ***p<0.001 vs. Day 0 (paired t-test within group).

3.9 Biochemical parameters

Serum alkaline phosphatase (ALP), elevated above normal at baseline across all groups consistent with hepatobiliary stress secondary to chronic fungal infection (Fouda *et al.,* 2021) declined highly significantly following treatment in all groups: Group I (162.40 ± 5.69 to 128.80 ± 4.31 U/l), Group II (158.30 ± 5.12 to 106.40 ± 3.06 U/l), and Group III (156.60 ± 4.04 to 110.00 ± 4.01 U/l). The

greatest absolute reduction was recorded in Group II (F1; 51.9 U/l), and was consistent with the well-documented hepatoprotective activity of *A. ilicifolius* phytoconstituents, including flavonoids and triterpenoids (Babu *et al.,* 2001; Rajamanickam *et al.,* 2016).

4. Discussion

The extract yield of 18.8% w/w was consistent with previously reported values for *A. ilicifolius* and indicates a good extraction yield of polar and semi-polar phytoconstituents, including alkaloids, flavonoids, saponins, tannins, and phenolic acids known to be soluble in methanol. The pH of both formulations (6.2-6.4) within the accepted range for canine skin ensures compatibility, maintenance of the skin surface environment, and may support antifungal activity of salicylic acid in the acidic microenvironment.

The presence of sugar, phenols, saponins, proteins, tannins, resins, and glycosides in the extract aligns with the earlier research report of Govindsamy and Arulpriya (2013), supporting the present findings and confirming the bioactive phytoconstituent profile responsible for the observed antifungal activity.

The higher distribution observed in young to middle-aged dogs (1-10 years: 67%) and a slight male preponderance are consistent with prior epidemiological studies. Breeds with dense undercoats, pendulous ears, or skin folds, such as Labrador Retrievers and German Shepherds, present known anatomical risk factors favouring fungal colonisation, explaining the breed-wise distribution observed.

The clinical features recorded, pruritus, alopecia, erythema, scaling/crusting, self-excoriation, and hyperpigmentation, correlate with established clinical profiles of dermatophytosis and *Malassezia* dermatitis. Anatomical predilection for the pinnae, ear canal, face, and ventral abdomen reflects the characteristic distribution pattern associated with these mycotic conditions. The predominance of *M. canis* among dermatophytes was in accordance with global epidemiological data reporting 50-98% *M. canis* prevalence in canine dermatophytosis. The high frequency of *Aspergillus* spp. and *C. albicans* in this study population reflects the humid climatic conditions of Maharashtra and is concordant with reports by Copetti *et al.* (2006) and Kalita *et al.* (2013).

The broad-spectrum activity of both formulations against filamentous fungi and yeasts supports a multitarget antifungal mechanism likely involving flavonoid-mediated membrane disruption, ergosterol biosynthesis inhibition, and oxidative stress induction, consistent with the mechanistic findings of Cushnie and Lamb (2005) and Susilawati *et al.* (2023). The dose-response relationship confirmed by F2 showing 39-49% more activity than F1 corroborates the results of Kalaskar *et al.* (2012) and Shojaee *et al.* (2023) in analogous herbal ointment research.

The hierarchical pattern ketoconazole > F2 (10%) > F1 (5%) validates concentration-dependent phytochemical efficacy and is consistent with published herbal antifungal research in veterinary dermatology. The recovery rate for ketoconazole (70%) aligns with systematic review data reporting 63-90% efficacy for topical ketoconazole in Malassezia-associated dermatological conditions (Choi *et al.,* 2019). The slightly lower absolute efficacy of the plant-based formulations compared to ketoconazole may reflect the non-specific multi-target mechanisms of phytochemicals, contrasting with the precise ergosterol biosynthesis inhibition of azoles, as well as concentration-dependent penetration kinetics through keratinised tissue. Nevertheless, the 10% formulation represents a clinically meaningful herbal alternative, particularly in settings constrained by resistance concerns, antifungal availability, or cost.

The significant reductions in TLC and eosinophil percentages across all treatment groups indicate effective resolution of systemic inflammation and attenuation of the T-helper 2 cell-mediated hypersensitivity response characteristically associated with mycotic skin disease. These haematological findings are consistent with those reported by Raheem-Ademola *et al.* (2012) and Anikar *et al.* (2022), who documented eosinophilia and leucocytosis in dermatophytosis-affected dogs that normalised following effective antifungal therapy. The absence of adverse haematological changes confirms the safety of topical ointment application.

The greatest absolute ALP reduction in Group II (F1; 51.9 U/l) is consistent with the well-documented hepatoprotective activity of *A. ilicifolius* phytoconstituents, including flavonoids and triterpenoids. The absence of hepatocellular toxicity (normal serum ALT and AST levels) stands in notable contrast to reports of itraconazole-associated ALT elevation in 12-42% of treated dogs. The clinically significant decline in albumin in Group III ($p=0.003$) underscores the importance of timely antifungal intervention to prevent protein catabolism associated with chronic mycotic disease. These findings are in line with Nafie *et al.* (2021) and Anikar *et al.* (2022), who reported normalisation of biochemical parameters following effective antifungal therapy in canine dermatophytosis.

5. Conclusion

This study provides compelling scientific evidence for the antifungal efficacy, anti-inflammatory activity, and systemic safety of topical *A. ilicifolius* extract ointment in naturally occurring canine mycotic dermatitis. Both 5% and 10% formulations demonstrated acceptable physicochemical properties, showing compatibility with canine skin. Along with the restoration of biochemical and haematological parameters. Collectively, these findings validate the traditional ethnopharmacological use of *A. ilicifolius* and establish it as a promising, cost-effective, multi-mechanistic phytotherapeutic alternative for canine mycotic dermatitis, particularly relevant in resource-limited settings and where antifungal resistance is a concern. Larger-scale, controlled clinical trials with long-term follow-up, molecular fungal speciation, and standardised phytochemical profiling are recommended to further characterise and optimise the therapeutic potential of this botanical formulation.

Acknowledgements

The authors are highly grateful to the Associate Dean, KNP College of Veterinary Science, Shirwal and Maharashtra Animal and Fisheries Sciences University, Nagpur, for providing all the facilities.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abad, M. J.; Ansuategui, M. and Bermejo, P. (2007). Active antifungal substances from natural sources. ARKIVOC, **7**:116-145.

Anikar, M. J.; Bhadesiya, C. M.; Gajjar, P. J.; Patel, V. A.; Dadawala, A. I.; Makwana, P. P. and Patil, D. B. (2022). Evaluation of therapeutic protocols containing different antifungal agents to treat dermatophytosis in dogs. The Pharma Innovation Journal, **11**:83-87.

Babu, B. H.; Shylesh, B. S. and Padikkala, J. (2001). Antioxidant and hepatoprotective effect of *A. ilicifolius*. Fitoterapia, **72**(3):272-277.

Babu, T. D.; Kuttan, G. and Padikkala, J. (2012). Cytotoxic and anti-tumour properties of certain taxa of *Umbelliferae* with special reference to *Centella asiatica* (L.) Urban. Journal of Ethnopharmacology, **48**(1):53-57.

Balouiri, M.; Sadiki, M. and Ibnsouda, S. K. (2016). Methods for *In vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, **6**(2):71-79.

Bernardo, F.; Lança, A.; Guerra, M. M. and Martins, H. M. (2005). Dermatophytes isolated from pet dogs and cats in Lisbon, Portugal (2000-2004). Revista Portuguesa de Ciências Veterinárias, **100**(553/554):85-87.

Bond, R.; Morris, D. O.; Guillot, J.; Bensignor, E. J.; Robson, D.; Mason, K. V. and Hill, P. B. (2020). Biology, diagnosis and treatment of *Malassezia* dermatitis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Veterinary Dermatology, **31**(1):27-e4.

Cafarchia, C.; Immediato, D.; Iatta, R.; Rambaldo, P.; Otranto, D. and Cirillo, R. (2012). Azole susceptibility of *M. pachydermatis* and *Malassezia* spp. isolated from dogs and cats with otitis externa and erythematous skin lesions. Medical Mycology, **50**(6):648-652.

Chen, T. A.; Halliwell, R. E. and Hill, P. B. (2002). Cutaneous *Malassezia* organisms and immune responses in dogs with atopic dermatitis. Veterinary Record, **151**(25):747-750.

Choi, F. D.; Juhasz, M. L. W. and Atanaskova Mesinkovska, N. (2019). Topical ketoconazole: A systematic review of current dermatological applications and future developments. Journal of Dermatological Treatment, **30**(8):760-771.

Copetti, M. V.; Santurio, J. M.; Cavalheiro, A. S.; Boeck, A. A.; Argenta, J. S.; Aguiar, L. C. and Alves, S. H. (2006). Dermatophytes isolated from dogs and cats suspected of dermatophytosis in Southern Brazil. Acta Scientiae Veterinariae, **34**(2):119-124.

Cowen, L. E.; Sanglard, D.; Howard, S. J.; Rogers, P. D. and Perlin, D. S. (2014). Mechanisms of antifungal drug resistance. Cold Spring Harbour Perspectives in Medicine, **5**(7):a019752.

Cushnie, T. P. and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, **26**(5):343-356.

Dubey, A. G.; Rode, A. M.; Dakshinkar, N. P.; Sanghai, A. and Bhojne, G. R. (2015). Comparative efficacy of different antifungal drugs in canine dermatomycosis. Indian Journal of Canine Practice, **7**:120-123.

Fisher, M. C.; Hawkins, N. J.; Sanglard, D. and Gurr, S. J. (2018). Worldwide emergence of resistance to antifungal drugs challenges human health and food security. Science, **360**(6390):739-742.

Fouda, A.; Abdel-Saeed, H.; Abdelgayed, S. S. and Abdou, O. M. (2021). Clinical, haemato-biochemical, and histopathological studies on some dermopathies in dogs. Advances in Animal and Veterinary Sciences, **9**(1):94-102.

Ghannoum, M. A. and Rice, L. B. (1999). Antifungal agents: Mode of action, mechanisms of resistance, and correlation of these mechanisms with bacterial resistance. Clinical Microbiology Reviews, **12**(4):501-517.

Govindasamy, C. and Arulpriya, M. (2013). Antimicrobial activity of *A. ilicifolius*: Skin infection pathogens. Asian Pacific Journal of Tropical Disease, **3**(3):180-183.

Guillot, J., & Bond, R. (2020). *Malassezia* yeasts in veterinary dermatology: an updated overview. Frontiers in cellular and infection microbiology, **10**:79.

Hill, P. B.; Lo, A.; Eden, C. A.; Huntley, S.; Morey, V.; Ramsey, S. and Sutton, C. (2006). Survey of the prevalence, diagnosis and treatment of dermatological conditions in small animals in general practice. Veterinary Record, **158**(16):533-539.

Kalaskar, P. S.; Karande, V. V.; Bannalikar, A. S. and Gatne, M. M. (2012). Antifungal activity of leaves of mangrove plant *A. ilicifolius* against *Aspergillus fumigatus*. Indian Journal of Pharmaceutical Sciences, **74**(6):575-579.

Kalita, C.; Dutta, T. C.; Phukan, A.; Goswami, S. and Saikia, G. K. (2013). Concurrent oral and topical management of mycotic dermatitis: A report of 18 canines. Intas. Polivet., **14**(2):342-344.

Khajure, P.V. and Rathod, J.L. (2010) Antimicrobial Activity of Extracts of *A. ilicifolius* Extracted from the Mangroves of Karwar Coast, Karnataka. Recent Research in Science and Technology, **2**:98-99.

Khinchi, R. K. (2019). Superficial pyoderma in canines in southern part of Rajasthan: A detailed epidemiological study. International Journal of Chemical Studies, **7**(3):2737-2740.

Kirk, N. M.; Vieson, M. D.; Selting, K. A. and Reinhart, J. M. (2021). Cytotoxicity of cultured canine primary hepatocytes exposed to itraconazole is decreased by pre-treatment with glutathione. Frontiers in Veterinary Science, **8**:621732.

Matos, J. C.; Guedes-Stehling, E.; Figueiredo, L. C.; Oliveira, L. A.; Oliveira, T. S.; Crotti, A. E. M. and Silva, M. L. A. (2022). *Acanthus ilicifolius*: A review of its phytochemistry and pharmacological properties. Phytochemistry Reviews, **21**(5):1405-1432.

Miller, W. H.; Griffin, C. E. and Campbell, K. L. (2013). Muller and Kirk's small animal dermatology (7th ed.).

Moriello, K. A.; Coyner, K.; Paterson, S. and Mignon, B. (2017). Diagnosis and treatment of dermatophytosis in dogs and cats: Clinical Consensus Guidelines of the World Association for Veterinary Dermatology. Veterinary Dermatology, **28**(3):266-e68.

Nafie, T.; Mahmoud, M. and Abdelkhalek, D. (2021). Clinical and laboratory studies of dermatophytosis-affected dogs in correlation to oxidative stress. Suez Canal Veterinary Medical Journal, **26**(1):17-26.

Natarajan, S.; Rameshbabu, V.; Ariharasudhan, S.; Hayavadana, J.; Rajpurohit, T. and Prakash, C. (2024). Antimicrobial properties of *Acanthus ilicifolius* and determination of phytochemical composition. Biomass Conversion and Biorefinery, **14**(23):29719-29726.

Newman, D. J. and Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products, **79**(3):629-661.

Patel, M.; Singh, J.; Ranjan, R. and Kumar, M. (2024). Epidemiological status and haemato-biochemical alterations in dogs with fungal dermatitis. The Pharma Innovation Journal, **13**(2):1847-1851.

Perlin, D. S.; Rautemaa-Richardson, R. and Alastruey-Izquierdo, A. (2017). The global problem of antifungal resistance: Prevalence, mechanisms, and management. The Lancet Infectious Diseases, **17**(12):e383-e392.

Pothiraj, G.; Balakrishnaraja, R. and Shanmugam, S. (2021). Evaluation of antibacterial and antifungal activity of *Acanthus ilicifolius* L. leaves extracts against clinically important microbes. Current Trends in Biotechnology and Pharmacy, **15**(2):166-174.

Raheem-Ademola, R. R.; Thomas, B. T.; Omolade, O. A. and Oluwadun, A. (2012). Investigation of haematological parameters and biochemical profiles in pupils with dermatophytosis. Report and Opinion, **4**(9):67-69.

Rajamanickam, E.; Gurudeeban, S.; Satyavani, K. and Ramanathan, T. (2016). Chemopreventive effect of *Acanthus ilicifolius* extract on modulating antioxidants, lipid peroxidation and membrane-bound enzymes in diethyl nitrosamine-induced liver carcinogenesis. International Journal of Cancer Research, **12**:1-16.

Sharma, R.; Sharma, R. and Jindal, N. (2021). Antifungal drug resistance: An overview. Indian Journal of Microbiology, **61**(2):131-140.

Shojaee, A.; Jahandideh, A.; Nasrollahi Omran, A.; Sohrabi Haghdoost, N. and Khosravi, M. (2023). Antifungal activity of *Gracilaria corticata* methanol extract against *Trichophyton, mentagrophytes*, *M.canis*, and *M. gypseum* on rat dermatophytosis models. Current Medical Mycology, **9**(1):14-20.

Singh, D. and Aeri, V. (2013). Phytochemical and pharmacological potential of *Acanthus ilicifolius*. Journal of Pharmacy and Bioallied Sciences, **5**(1):17-20.

Singh, R.; Beigh, S. A.; Soodan, J. S.; Tikoo, A. and Tantaray, H. (2012). Clinico-epidemiological studies in canine dermatitis. Indian Journal of Canine Practice, **4**(2):96-99.

Soares, B. V.; Morais, S. M.; Fontenelle, R. O.; Brito, E. H.; Queiroz, V. A.; Castro, C. M. S. and Rocha, M. F. (2015). Antifungal activity of plant extracts against *M. canis* and *Candida* spp. strains. Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas, **14**(4):263-272.

Susilawati, E.; Wahyuni, S. and Khairunisa, S. Q. (2023). Flavonoids as effective anti-Candida agents: A review. Journal Sains Farmasi dan Klinis, **10**(2):126-133.

Tripathi, Y. C.; Malviya, M.; Singh, V. K. and Kamboj, V. P. (2024). Pharmacological aspects of mangrove plants: A comprehensive review. Heliyon, **10**(2):e23909.

Vijayakaran, K.; Ranganathan, V. and Kumar, P. S. (2020). Antifungal activity of selected medicinal herbs against canine dermatophytosis. Journal of Entomology and Zoology Studies, **8**(6):1618-1622.

Villar, M.; Martin-Latil, S.; Gortazar, C.; Rogniaux, H.; Sansonetti, P. J.; Velge, P. and Gomes-Solecki, M. (2023). Phytotherapy for infectious disease: A valuable strategy to control antimicrobial resistance. Frontiers in Microbiology, **14**:1148940.

Citation **A.S. Khairmode, V.V. Karande, B.C. Ghumare, U.M. Tumlam, B.N. Ambore, S.N. Jadhav and M. B. Bukke (2026). Evaluation of the antifungal efficacy of *Acanthus ilicifolius* L. extract ointment in canine mycotic dermatitis. Ann. Phytomed., 15(1):720-726, http://dx.doi.org/10.54085/ap.2026.15.1.64.**