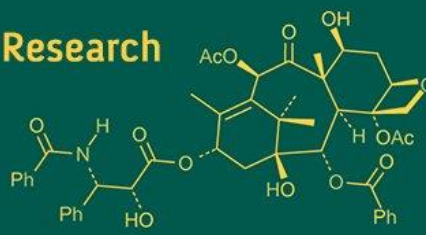
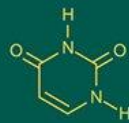
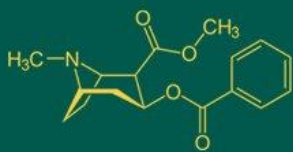


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From resistance to remedies: black turmeric (*Curcuma caesia*) against canine *Staphylococcus aureus* dermatitis

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Abstract

Canine dermatitis is a complex and multifactorial inflammatory skin disease characterized by erythema, oedema, alopecia, pruritus, and cutaneous lesions. *Staphylococcus aureus* frequently serves as critical opportunistic bacteria that colonize on the compromised epidermal barriers of skin. This review underscored the quick presence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains driven by the *mecA* and *mecC* genes and therefore, results in limiting veterinary therapeutic choices as well as serves as major zoonotic threat to human health. Conventional bacteriological identification relies on selective growth on Mannitol Salt Agar (MSA), phenotypic golden pigmentation, and key biochemical assays (coagulase-positive, catalase-positive, and DNase-positive). Molecular confirmation was performed by targeting the highly conserved 16S rRNA gene (a ~1,500 bp amplicon) using a single-plex PCR assay. Crude extracts and essential oils of *Curcuma caesia* rhizomes possess diverse therapeutic properties. Pharmacological evaluations demonstrate significant antimicrobial, antioxidant, analgesic, anti-inflammatory, hepatoprotective, and antimutagenic activities, primarily mediated through TNF-alpha and NF-kappa-B pathway down-regulation. Additionally, *C. caesia* exhibits CNS depressant, anticonvulsant, cytotoxic, anthelmintic and anticancer effects against liver malignancy and Ehrlich's Ascites Carcinoma have been highlighted. This current review also accentuated on the phytochemicals such as flavonoids, phenolics, and essential oils contribution as potent antibacterial efficacy against multidrug-resistant pathogens like *S. aureus* and for the effective therapeutic potential in canine dermatitis lesions, chronic wounds and supporting herbal veterinary dermatological therapies. This review summarizes about future prospects of phytochemicals in the way of pharmacologically prudence and therapeutically proven especially in the fighting of inevitable antimicrobial resistance and backings the core concept of world health approach.

Keywords: Antimicrobial resistant, black turmeric, canine dermatitis, *Curcuma caesia*, methicillin resistance *Staphylococcus aureus* (MRSA), phytochemicals, and 16S rRNA

1. Introduction

Canine dermatitis is an inflammatory skin disorder characterized by erythema, pruritus, edema, alopecia, and cutaneous lesions. It frequently leads to secondary bacterial infections that delay wound healing and compromise the overall health of the animal. Among the bacterial pathogens, *Staphylococcus aureus* is considered one of the important opportunistic bacteria associated with canine dermatitis. It inhabits on the skin and mucous membranes but infection starts due to breach of skin barrier and weak immunity.

In a retrospective study conducted during 2003-2004, antimicrobial resistance was documented among *Staphylococcus aureus*, *S. intermedius*, and *S. schleiferi* isolates obtained from 749 clinical dermatitis lesions in small companion animals (Morris *et al.*, 2006)^[44]. The emergence of antibiotic-resistant *Staphylococcus aureus* strains poses a dual crisis, severely impacting veterinary clinical care while presenting a major threat to human health.

Medicinal plants have been used for centuries in traditional healthcare systems, and contemporary scientific researchers have increasingly focused on validating these herbal remedies. Therefore, Black turmeric (*Curcuma caesia* Roxb.) has attracted significant attention for its wide range of therapeutic potentials. It belongs to the family Zingiberaceae, bluish-black rhizome with characteristic aroma and along with that its phytochemical composition includes flavonoids, phenolic compounds, alkaloids, terpenoids, curcuminoids,

and essential oils, together exhibit antimicrobial, antioxidant, anti-inflammatory, wound healing properties, skin disorders, inflammation, respiratory ailments, and microbial infections. Considering the increasing problem of antimicrobial resistance and the growing interest in herbal therapeutics, black turmeric appears to be a promising candidate for managing bacterial skin infections in dogs. However, scientific evidence regarding its therapeutic efficacy against *Staphylococcus aureus*-associated canine skin dermatitis remains limited. Therefore, this review highlights the isolation, identification, and molecular characterization of *Staphylococcus aureus* in canine dermatitis, alongside the therapeutic and antimicrobial potential of black turmeric (*Curcuma caesia*) extract. Ultimately, this combination provides valuable insights for developing safe, effective, economical, and sustainable plant therapeutics to manage canine bacterial skin infections.

2. Canine Dermatitis

Canine atopic dermatitis is one of the most common inflammatory dermatoses encountered in modern small animal veterinary practice. As highlighted by Saridomichelakis and Olivry (2016) [59], its clinical manifestation characterized by cutaneous inflammation and intense pruritus is driven by a complex, multifactorial aetiology. They further documented that chronic pruritus is frequently complicated by secondary bacterial folliculitis, *Malassezia* yeast overgrowth, and concurrent dietary hypersensitivities. But in the earlier studies by the Patrick *et al.* (2015) [49] recorded that, canine dermatitis lesions were genetically predisposed, inflammatory and pruritic skin disease and thereby, variations in the presentations with respect to the extent of the skin disorder, due to genetic factors, stage of the disease, secondary infections, as well as resemblance to other non-atopic related skin diseases.

Several recent studies have also emphasized non-infectious aetiologies responsible for canine dermatitis conditions. Yvonne and Charli D. (2024) [73] investigated atopic dermatitis (AD), a common inflammatory and pruritic allergic skin disease affecting both humans and dogs worldwide. According to their findings, the pathogenesis of AD is multifactorial and immunologically complex, involving genetic predisposition, disruption of the natural skin barrier, alterations in the skin microbiome, dysregulation of the immune system, and allergic sensitization. This review presents comprehensive facts on the occurrence, clinical impact, and complex immunological connections underlying atopic dermatitis.

3. Bacterial Etiology of Canine Dermatitis

In the earlier studies, it has been consistent that, the bacterial dermatitis is widely understood to be secondary to preexisting cutaneous breach of natural immunity. As established by Scott *et al.* (2001) [62], structural and functional damage to the skin barrier driven by underlying factors such as allergies, ectoparasites, mechanical trauma, or endocrine dysfunction provide a favourable environment for opportunistic bacterial invasion. Guardabassi *et al.* (2015) [23] emphasized that the indiscriminate use of antibiotics has accelerated the emergence of multidrug-resistant bacterial strains in companion animals, rendering rigorous antimicrobial stewardship essential for effective veterinary management. Bacterial skin infections in companion animals have historically been attributed primarily with *Staphylococcus*

intermedius and *Staphylococcus aureus*. However, Morris *et al.* (2006) [44] emphasized that newly identified staphylococcal organisms might have been combined in the companion animal pyoderma. Furthermore, the authors also noted a rising incidence of methicillin-resistant *Staphylococcus* (MRS) strains, coupled with the potential risk of zoonotic transmission to humans; dermatoses in small companion animal practices thus represent a major route of transmission for this zoonotic concern. In addition to this study, Boost *et al.* (2011) [7] demonstrated that healthy domestic dogs frequently harbor pathogenic *Staphylococcus species* without displaying noticeable clinical disorders with respect to the dermatitis. Consequently, these subclinical carriers act as unseen environmental reservoirs, facilitating the persistence and dissemination of the infections towards the profound zoonotic implications of this silent carriage. Further studies on microbiological profile of canine pyoderma revealed a clear difference between resident and transient pathogens and on this aspect Bannoehr and Guardabassi (2012) [6] identified *Staphylococcus pseudintermedius* as the primary opportunistic agent responsible for canine skin infections but, *Staphylococcus aureus* was found to be isolated less often from canine lesions, but remained clinically significant for its zoonotic potential.

Tong *et al.* (2015) [67] elucidated that the bacterium utilizes a sophisticated combination of cytotoxic toxins, extracellular enzymes, and advanced biofilm-forming capabilities. These coordinated virulence factors allow the organism to competently colonize disrupted cutaneous barriers, escape active host immune responses, and establish dermatitis lesions. Morris *et al.* (2017) [43] reported that bacterial dermatitis causes inflammation of the skin and hair follicles, resulting in erythema, pruritus, pustules, crusting, and alopecia. They further emphasized that early diagnosis significantly improves treatment outcomes.

In continuation with these observations, earlier microbiological studies have revealed that *Staphylococcus pseudintermedius* remains the principal bacterial species isolated from canine pyoderma, while *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Streptococcus species* are also recovered from affected dogs (Hillier *et al.*, 2014) [28].

To broaden the discussion of bacterial aetiologies beyond *Staphylococcus species*, Faccin *et al.* (2023) [17] identified *Mycobacterium spp.*, *Nocardia spp.*, and *Actinomyces spp.* as the primary drivers of these deep, destructive inflammatory conditions, resulting in granulomatous or pyogranulomatous dermatitis.

Loeffler *et al.* (2025) [38] reported that staphylococci are the primary isolates in canine pyoderma, emphasizing that these infections are typically secondary to an underlying disease and require identification of the primary cause for successful resolution.

4. Role of *Staphylococcus aureus* in canine dermatitis

Scott *et al.* (2001) [62] recognized that bacterial pyoderma is among the most prevalent dermatological conditions in canine practice. The authors noted that *Staphylococcus aureus* and related staphylococcal species readily invade compromised skin, resulting in characteristic inflammation, papules, pustules, and crusts; crucially, this colonization is heavily facilitated by disruptions to the epidermal barrier and weakened host immune defenses. Nuttall *et al.*, (2013) [46] and

Ravens *et al.*, (2014)^[53], demonstrated Gram staining of the purified cultures revealed Gram-positive cocci arranged in irregular, grape-like clusters, consistent with the staphylococcal morphology. Rana *et al.* (2024)^[52] and Debbarma *et al.* (2026)^[14] isolated the *S. aureus* based upon the colony characterization on nutrient agar and Mannitol Salt Agar (MSA) and it revealed medium-sized, circular colonies with intact margins, a convex raise, and a smooth, buttery texture. On MSA, colonies revealed creamy-to-golden pigmentation, a key phenotypic indicator used to identify the isolates as *S. aureus*. And in addition to this, yellowish coloured selective growth conditions facilitated by Mannitol Salt Agar (MSA) confirmed the positive indicator for the *Staphylococcus aureus* and parallel findings were also reported by Hadi and Alabbas (2023)^[26].

Rusenova and Rusenov (2017)^[57] isolated and identified *S. aureus* based on biochemical profiling, revealing that all isolates were coagulase-positive; further tests demonstrated catalase-positive, oxidase-negative, mannitol-fermenting, DNase-positive, urease-positive, nitrate-reducing, and fermentative reactions on carbohydrate media.

The emergence of hyper-virulent, community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains has escalated public health risks by causing severe infections in healthy populations outside clinical settings (Chambers, 2005)^[9]. Phenotypically distinct, strains are now the predominant cause of skin and soft tissue infections managed in U.S. emergency departments (Moran *et al.*, 2006)^[42]. Although the mechanisms of acquired immunity against persistent staphylococcal pathologies remain obscure, host innate immunity critically dictates overall infection outcomes (Komatsuzawa *et al.*, 2006)^[36].

Ihrke and Gross (2006)^[30] characterized canine pyoderma as a secondary consequence of predisposing dermatopathies, including hypersensitivities, ectoparasitism, and endocrinopathies. The authors noted that staphylococci act as opportunistic pathogens, proliferating rapidly upon epidermal barrier disruption to cause persistent cutaneous lesions.

In continuation to this above observation, Ruscher *et al.* (2009)^[56] also recorded in the earlier study that, *S. aureus* was identified as the principal aetiological bacterial agent associated with canine dermatitis as opportunistic pathogens. Fazakerley *et al.* (2009)^[19] evaluated staphylococcal colonization across multiple anatomical sites in healthy and atopic dermatitis dogs, and isolated *S. pseudintermedius*. Conversely, *Staphylococcus aureus* colonization was found to be low. In respect to the involvement of *S. aureus* in the canine dermatitis, Boost *et al.* (2011)^[7] further established that companion animals can harbor these bacteria asymptomatically, serving as obscure reservoirs for recurrent infections and contributing to household transmission.

Bannoehr and Guardabassi (2012)^[6] reviewed the epidemiology of canine dermatological disorders and established that, *Staphylococcus pseudintermedius* remains the predominant pathogen, and *Staphylococcus aureus* was progressively isolated from pyoderma cases of dogs. Furthermore, Morris *et al.* (2017)^[43] noted that *S. aureus* elaborates a set of virulence factors including toxins, exoenzymes, and biofilms that significantly enhance epithelial adhesion, tissue invasion, and persistent colonization within the dermis, ultimately prolonging pathologies and undermining therapeutic outcomes.

Loeffler and Lloyd (2010)^[39] reviewed the epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in companion animals and this asymptomatic carriage highlights a critical zoonotic risk, underscoring the necessity for precise bacterial isolation and identification.

Clinically, Nuttall *et al.* (2013)^[46] emphasized that staphylococci remain the leading cause of canine pyoderma, maintaining that bacterial culture, species identification, and antimicrobial susceptibility testing are vital prerequisites for effective therapeutic management. To optimize these diagnostic outcomes, Ravens *et al.* (2014)^[53] evaluated various sampling methodologies for canine superficial pyoderma, concluding that meticulous sample collection paired with robust phenotypic and biochemical identification significantly improves the recovery and accurate diagnosis of *Staphylococcus* species.

Hill and Imai (2016)^[27] reviewed the immunopathogenesis of canine staphylococcal pyodermas, describing the specific virulence factors enable *Staphylococcus aureus* to colonize epidermal surfaces, evade host immunity, and establish chronic infections. To improve clinical detection of these persistent strains, Chaudhary *et al.* (2023)^[11] compared conventional bacteriological assays with molecular techniques, demonstrating that polymerase chain reaction (PCR) assays targeting the 16S rRNA gene (Species specific) for the rapid and highly accurate confirmation.

The critical nature of such precise diagnostics is underscored globally by Abdullahi *et al.* (2022)^[1], whose systematic review of *S. aureus* lineages in dogs highlighted a worrying intersection of high prevalence, complex virulence factors, and escalating antimicrobial resistance, cementing the pathogen's zoonotic importance and the necessity for continuous epidemiological surveillance. dogs presenting with dermatological lesions—including erythema, pustules, papules, crusts, alopecia, pruritus, and purulent exudate—identified a bacterial profile as described by Tanveer *et al.* (2024)^[65] in a systematic review and meta-analysis of canine pyoderma and otitis externa cases in Korea, reported *Staphylococcus* and *Pseudomonas* as the predominant bacteria associated with canine dermatitis.

However, PCR-based genotyping in several previous studies identified coagulase-positive *Staphylococcus pseudintermedius*, rather than *S. aureus*, as the predominant staphylococcal species associated with canine pyoderma by Gonzalez-Dominguez *et al.* (2020)^[22].

For molecular detection of *S. aureus*, Chavda *et al.* (2025)^[12] targeted the highly conserved 16S rRNA gene using simplex PCR, yielding an amplicon size of 1500 bp. Further, this result aligns with the approach taken by Rosa *et al.* (2018)^[55] in the earlier studies and apart from that, Chaudhary *et al.* (2023)^[11] demonstrated PCR assays targeting the 16S rRNA and ensured the confirmation of *S. aureus*.

5. Antimicrobial Susceptibility Patterns of Bacterial Pathogens Isolated From Canine Dermatitis

In order to draw a comparison in the line of discussion with the antibiogram of *Staphylococcus* organisms the investigations conducted by the earlier researchers revealed that, cephalexin, were found to be the most effective antimicrobials and further demonstrated its antimicrobial effectiveness by achieving high concentrations within cutaneous tissues along with excellent tolerability (Papich *et al.*, 2013)^[48].

The International Society for Companion Animal Infectious Diseases (ISCAID) guidelines published by Hillier *et al.* (2014)^[28] and recommended that, cephalexin, cefadroxil, and amoxicillin-clavulanate as useful and foremost systemic therapies for simple canine pyoderma and authors also emphasized equally, higher-tier agents like fluoroquinolones strictly set aside for cases supported by definitive investigative culture and susceptibility profiles.

5.1. Methicillin Resistance *Staphylococcus aureus* (MRSA): A comprehensive study conducted by Wedley *et al.* (2014)^[71] in the United Kingdom screened 724 dogs from 87 veterinary practices across mainland UK for carriage of *Staphylococcus* spp., revealing that isolates harbouring the *mecA* gene exhibited strong resistance to methicillin and related antimicrobial agents

In addition to the earlier observations on the findings of antibiogram profiles of bacterial isolates from the canine skin infections, Guardabassi and Prescott (2015)^[23], highlighted about the rising prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) gradually invalidates empirical selections of penicillin and conventional beta-lactam antibiotics. In a study of clinical staphylococcal isolates from a veterinary dermatology clinic in Belgrade, Prosić *et al.* (2024)^[51] identified a 29.4% MRSA prevalence rate and it was validated by using a combination of phenotypic MIC values, PBP2a protein detection, and multiplex PCR targeting the *mecA* and *mecC* genes. In line with similar studies, González-Domínguez *et al.* (2020)^[22] demonstrated that *Staphylococcus species* isolated from canine superficial pyoderma (CSP) carried the *mecA* gene, confirming methicillin resistance among the isolates. Therefore, molecular characterization of bacteria causing dermatological conditions is an essential step, given their high-level resistance to commonly used antimicrobials.

Further studies in the areas like antibiogram profile of canine dermatitis cases, Weese *et al.* (2015)^[72] advocated for the restriction of high-tier agents such as fluoroquinolones and glycopeptides to alleviate the rushing of antimicrobial resistance. This mandate for diagnostic precision was also underscored in chronic cases by Loeffler *et al.* (2018)^[40], who observed that persistent canine pyoderma were repeatedly showing resistant staphylococcal strains and therefore, authors validated about the bacterial culture and susceptibility testing are obligatory before deploying non-conventional antimicrobials such as gentamicin, ceftriaxone, or enrofloxacin.

6. Extraction and Therapeutic Evaluation of Black Turmeric (*Curcuma caesia*) and Its Antimicrobial Potentials

Curcuma caesia Roxb., commonly designated as black turmeric, a highly valued medicinal plant of Zingiberaceae family and characterized by its distinct bluish-black rhizomes, camphoraceous aroma has increasingly become the focus of modern pharmacological validation due to its rich profile of bioactive secondary metabolites, including curcuminoids, essential oils (predominantly camphor, ar-turmerone, and 1,8-cineole), flavonoids, and phenolics (Ibrahim *et al.*, 2023)^[29].

According to Fathima *et al.* (2015)^[18], the rhizomes of *Curcuma caesia* were shade-dried and subsequently pulverized into a fine powder using an electrical processor. A

100-gram portion of the powdered biomass underwent hot continuous extraction via a Soxhlet apparatus utilizing 500 mL of absolute ethanol. The resulting ethanolic extract was concentrated through distillation, evaporated to dryness under vacuum, and stored in a desiccator inside a refrigerator prior to pharmacological evaluation.

Reenu *et al.* (2015)^[54] investigated the extraction of bioactive constituents from fresh and dried rhizomes of *Curcuma caesia*. Approximately 50 g of each rhizome sample underwent sequential exhaustive extraction using 500 mL each of hexane, petroleum ether, benzene, chloroform, ethyl acetate, methanol, and water, spanning a gradient of increasing polarity. Following yield quantification, the dried extracts were stored at 4°C for the evaluation of its antioxidant properties. Chaturvedi *et al.* (2020)^[10] demonstrated that the antimicrobial efficacy of *C. caesia* is influenced by extraction methods, with methanolic extracts yielding superior activity. This finding highlighted the importance of solvent selection in phytochemical research. Sawant *et al.* (2014)^[61] demonstrated the methanolic extract of *Curcuma caesia* rhizomes (MECC) using soxhlet apparatus for 16 hours and then filtered at room temperature. The filtrate was dried on tray dryer at 40°C (yield - 9.2% w/w). The dried extract was dissolved in distilled water containing 2% gum acacia to prepare the drug extract and are being used for pharmacological evaluation of the analgesic and anti-inflammatory potential of a methanolic extract of *Curcuma caesia* rhizomes in animal models.

Tamang (2022)^[64] described the phytochemical profiles and that, showed the rhizome harbors a dense matrix of bioactive secondary metabolites, including alkaloids, terpenes, tannins, flavones, flavonoids, steroids, glycosides, and anthraquinones. Further studies revealed that, the rhizomes exhibit profound carminative and smooth muscle relaxant activities, alongside documented efficacy in managing respiratory ailments like asthma, neurological conditions such as epilepsy, febrile illnesses, and chronic inflammatory conditions (Sasikumar, 2005; Arulmozhi *et al.*, 2006 and Gantait *et al.*, 2011)^[60,5,20].

Paudel *et al.* (2024)^[50] highlighted the pharmacological value of *Curcuma caesia* Roxb. (Black turmeric), as an indigenous rhizomatous herb are being traditionally utilized by indigenous communities across Nepal and India. Phytochemical profiling of the plant's leaves and rhizomes identified key bioactive monoterpenes and sesquiterpenes, including camphor, 1,8-cineole, ar-curcumen, beta-elemene, borneol, bornyl acetate, and alpha-terpineol. The authors demonstrated that these secondary metabolites confer distinct antibacterial, antioxidant, anti-inflammatory, and antineoplastic properties. Given this multi-targeted bioactivity, *C. caesia* presents a viable, plant-based alternative to synthetic compounds currently employed in oncology, wound care management, infectious disease control, and immunomodulation.

Chadalavada *et al.* (2017)^[8] documented the extensive ethnomedicinal and pharmacological profile of *Curcuma caesia* rhizomes, noting their widespread use in treating respiratory, dermatological, and systemic disorders. Traditionally, the rhizomes are utilized to alleviate respiratory ailments such as coughs, colds, and pneumonia in children, as well as asthma and bronchitis in adults. Topically, a paste of the fresh rhizomes is applied to facilitate the rapid healing of wounds, cuts, and chronic sores, and is used as a remedy for leukoderma and rheumatic arthritis.

Internally, small doses of the rhizome paste are administered to manage menstrual disorders, expel gastric gas, and treat conditions like hemorrhoids (piles) and splenomegaly (enlargement of the spleen). Furthermore, the authors highlighted its traditional application against severe conditions including epilepsy, leprosy, gonorrheal discharge, tumors, and HIV/AIDS. Pharmacologically, the rhizomes which are rich in starch and a camphor-heavy essential oil exhibit potent antioxidant, antitumor, and smooth muscle relaxant activities. Additionally, the volatile oil possesses robust antibacterial properties, demonstrating marked inhibition against Gram-positive pathogens such as *Staphylococcus aureus* and *Bacillus subtilis*.

Vineela *et al.* (2017)^[70] demonstrated that the essential oils of *Curcuma caesia* composed of rich mixtures of monoterpenes, sesquiterpenes, and aliphatic hydrocarbons serve as potent antimicrobial agents. Specifically, the ethanolic extracts of *C. caesia* (EECC) exhibit pronounced antibacterial efficacy against *Staphylococcus aureus*, a characteristic strongly linked to the presence of endogenous phenolic compounds. In evaluation of radical scavenging metrics (DPPH and hydroxyl radical assays), non-enzymatic extracts within the *Curcuma* genus consistently show superior free radical scavenging capacities compared to their enzymatic counterparts. Furthermore, heat-stable proteins isolated via aqueous Soxhlet extraction from *C. caesia* rhizomes display robust antioxidant functions along with significant anti-inflammatory activity when validated using a carrageenan-induced rat paw edema model. The volatile profile causing these therapeutic attributes has been extensively mapped.

Further investigation on foundational phytochemical screenings of *C. caesia* using diverse solvents including n-hexane, petroleum ether, benzene, chloroform, ethyl acetate, methanol, and water have confirmed that the underlying biomass is abundant in systemic secondary metabolites, notably alkaloids, phenols, phytosterols, terpenoids, glycosides, saponins, quinones, amino acids, and flavonoids (Sarangthem and Haokip, 2010; Paliwal *et al.*, 2011; Donipati and Sreeramulu, 2015 and Mukunthan *et al.*, 2017)^[58, 47, 16, 45].

The extraction yield and physical state of the raw material significantly modulate these therapeutic outcomes and in continuation with this aspects Reenu *et al.* (2015)^[54] examined these extraction dynamics using the DPPH free radical scavenging assay across diverse solvent fractions and revealed that, the highest antioxidant activities were recovered in the chloroform (67.76%) and methanol (67.36%) extractions method, while the lowest was recorded in the hexane fraction (23.43%). But the extracts obtained from dried rhizomes exhibited vastly superior free radical scavenging capacities, peaking at 92.98%, 91.48%, and 73.07% within the chloroform, ethyl acetate, and methanol fractions, respectively.

7. In Vitro Evaluation of the Therapeutic Potentials of Crude Extracts of *Curcuma caesia* Rhizome

As evidenced by numerous studies, the *in-vitro* evaluation of crude *Curcuma caesia* rhizome extracts revealed substantial efficacy in combination with conventional antimicrobial agents. In a comparative study, Kaur *et al.* (2011)^[34] appraised the analgesic and antipyretic efficacy of various extracts from *C. caesia* and *Curcuma amada* rhizomes utilizing a chemical model of acute pain and brewer's yeast-

induced hyperthermia in rats. Their findings revealed that while both species possessed therapeutic properties, *C. caesia* elicited a lower analgesic potential and antipyretic response compared to *C. amada*. Expanding on alternative therapeutic fractions, Angel *et al.* (2012)^[4] isolated a heat-stable protein from the rhizomes of *C. caesia* via aqueous Soxhlet extraction and on *in-vivo* evaluation; the protein fraction demonstrated significant antioxidant capabilities and exhibited potent anti-inflammatory activity at a therapeutic dosage of 100 mg/kg within a carrageenan-induced rat paw edema model system.

Karmakar *et al.* (2011)^[33] assessed the neuropharmacological properties of *Curcuma caesia* rhizome extracts in murine models, and documented a significant reduction in basal locomotor activity that indicates a pronounced central nervous system (CNS) depressant effect. Furthermore, the methanolic extract significantly antagonized pentylenetetrazol (PTZ)-induced convulsions, validating its potential as an anticonvulsant and sedative agent.

In view of the assessment of therapeutic potentials of *C. caesia*, Das *et al.* (2012)^[13] reported about the gastro protective and anti-ulcerogenic potential of the ethanolic extract of *Curcuma caesia*. Furthermore, an ethanolic *C. caesia* extract significantly reduced acid output, gastric juice volume, and the overall ulcer index in aspirin-induced by using experimental rat models.

In another study, a methanolic extract of *Curcuma caesia* demonstrated strong antitumor potential against Ehrlich's Ascites Carcinoma (EAC) in murine models. The rhizome extract exhibited direct *in-vitro* cytotoxicity against EAC cells while significantly reducing tumor volume, tumor weight, and viable cancer cell counts *in vivo* (Karmakar *et al.*, 2013)^[32].

Complementing these findings, Hadem *et al.* (2014)^[25] evaluated the hepatoprotective and anticancer properties of *C. caesia* against diethylnitrosamine (DEN)-induced hepatocarcinogenesis. While DEN administration severely elevated the activities of key serum toxicity marker enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and acetylcholinesterase (AChE) and induced preneoplastic nodules, and evaluation of anti-tumor efficacy of *C. caesia* revealed that, it effectively down-regulated these enzyme levels. Moreover, *C. caesia* treatment prevented the depletion of both enzymatic and non-enzymatic hepatic antioxidant defense systems, thereby demonstrating potent chemo preventive efficacy against chemically induced liver malignancy.

Devi *et al.* (2015)^[15] evaluated the antimutagenic potential of *Curcuma caesia* rhizome extracts against the indirect-acting mutagen cyclophosphamide and observed that, the ethanolic extract drastically suppressed mutagenicity by 90% at the highest tested concentration in *Salmonella typhimurium* strains infection in the presence of metabolic activation, and thereby demonstrating a robust protective effect against chemotherapeutic-induced genotoxicity. In another study, the anti-inflammatory, antiproliferative, and anticarcinogenic progression pathways of *C. caesia* have been linked to transcriptional regulation in murine models. Because tumor necrosis factor-alpha (TNF-alpha)-induced cell proliferation is fundamentally mediated by the activation of nuclear factor kappa B downstream signaling, down-regulating this pathway provides significant therapeutic value as reported by Hadem *et al.* (2016)^[25] and further observations demonstrated that *C. caesia* treatments markedly lowered

serum TNF-alpha levels and inhibited NF-kappa B DNA-binding activity, resulting in a marked reduction in both tumor onset and progression.

Shakya *et al.*, (2015) ^[63] studied about different fraction of *Curcuma caesia* for its antibacterial and cytotoxic activity. The studied revealed the potent cytotoxic effect of petroleum ether extract, diethyl ether extract, chloroform extract and methanol extract showing LC50 value of 18.923mcg/ml, 1.086 mcg/ml, 45.289 mcg/ml and 100 mcg/ml respectively, less than 1000 mcg/ml. The antibacterial activity has not shown by any extract because of the absent of tannins in all the extracts of it. Chadalavada and Budala (2017) ^[8] studied the anthelmintic activity of three different extracts of *Curcuma caesia* Roxb. Among the various extraction methods evaluated, the ethanolic extract of *Curcuma caesia* Roxb displayed the highest anthelmintic efficacy, causing rapid paralysis and death of earthworms at a concentration of 100 mg/mL.

8. Antimicrobial Activity of Crude Extract of *Curcuma caesia*:

A comprehensive studies on the anti-microbial efficacy of *Curcuma longa* by Teow *et al.* (2016) ^[66] established that, the extracted compound Curcumin showed significant susceptibility against *Staphylococcus aureus*, and demonstrating strong potency against both methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) and apart from that, the diverse mechanisms of actions were also documented in this investigation, such as disruption of bacterial membrane integrity, inhibition of biofilm development, when combined with conventional antibiotic therapies.

In case of evaluation study of antimicrobial potential of *C. caesia* by Khuntia *et al.* (2023) ^[35] documented that, the bioactivity of essential oils of *C. caesia* revealed promising antibacterial activity against clinical multidrug-resistant (MDR) bacterial isolates and this investigation established that *C. caesia* essential oil possesses broad-spectrum antimicrobial properties, positions it as a viable candidate for combating resistant pathogens, and confirms its utility as an alternative therapeutic agent in contemporary pharmacology. In continuation to this findings, Garnayak (2023) ^[21], demonstrated the *in-vitro* and *in-vivo* anti-microbial properties of methanolic and ethanolic extracts of rhizomes of *C. caesia* against multi drug resistance bacteria like *Escherichia coli* and *Staphylococcus aureus* in mouse model. This review conclusively summarized the phytochemical profile and pharmacological characteristics of *Curcuma caesia* Roxb., emphasizing that its multifarious matrix of flavonoids, essential oils, phenolics, and camphor drives its broad antibacterial efficiency. The findings further underline the plant's ethno-medicinal significance in treating dermatological disorders, chronic wounds and backup its development as a besieged natural antimicrobial agent.

9. Recent Advances in Herbal Therapeutics for Canine Dermatitis:

In recent years, pharmaceutical research has increasingly focused on exploring novel avenues in phytochemistry, investigating bioactive compounds within medicinal plants and functional foods as safer therapeutic alternatives. Recent advancements in characterizing these phytochemicals offer promising pathways for discovering potent antimicrobial, anticancer, and antidiabetic agents. Tresch *et al.* (2019) ^[68] conducted a broad systematic review evaluating the clinical and experimental evidence for

botanical topicals in veterinary dermatology and highlighted those specific medicinal plants most notably *Calendula officinalis* (marigold), *Matricaria chamomilla* (chamomile), and *Salvia officinalis* (sage) exhibit potent, scientifically validated antimicrobial, anti-inflammatory, and tissue-regenerative properties.

Marsella *et al.* (2021) ^[41] discussed recent advancements in the management of canine atopic dermatitis, emphasizing that complementary herbal therapies possessing anti-inflammatory and antioxidant properties can effectively support conventional protocols. These botanical interventions help attenuate cutaneous inflammation, alleviate oxidative stress, and decrease the recurrence of clinical lesions.

In a comprehensive literature review, van Amersfort *et al.* (2023) ^[69] evaluated the evidence base for nutraceuticals and herbal products in canine dermatological immune-mediated inflammatory diseases and further study revealed that plant-derived bioactive compounds including polyphenols, flavonoids, and omega-rich botanicals exhibit distinct immunomodulatory and anti-inflammatory properties, but the available literature provides only minor clinical evidence regarding their efficacy in improving outcomes in cases of dogs with allergic and inflammatory skin disorders.

Jacobs *et al.* (2025) published an integrative review highlighting the expanding application of herbal medicines and essential oils in the management of canine skin diseases and documented that, the botanical extracts possess potent antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. These bioactive represent promising natural alternatives for treating superficial pyoderma while concurrently mitigating clinical reliance on conventional antibiotics.

Canine dermatitis frequently encounter with the secondary bacterial infection predominantly by *Staphylococcus*. The several strains of *S. aureus* display the significant level of multidrug-resistant and methicillin-resistant, denotes a increasing challenge in veterinary dermatological diseases and is associated with significant zoonotic consequences. The escalating failure of conventional antibiotic regimens underscores the urgent need for alternative, sustainable therapeutic approaches. *Curcuma caesia* Roxb. (Black turmeric) offerings itself as a convincing herbal solution, owed to its rich phytochemical composition of curcuminoids, essential oils, flavonoids, and phenolics. Current *In Vitro* and *in vivo* indication supports its multi-dimensional therapeutic potential and that covering antimicrobial, anti-inflammatory, antioxidant, wound-healing, and immunomodulatory activities along with encouraging synergistic effects when combined with standard and conventional antimicrobial therapies.

However, in spite of this considerable pharmacological basis, scientific endorsement of *C. caesia*'s efficacy against *S. aureus*-associated canine dermatitis remains scarce. Future researches must be highlighted upon the molecular characterization of resistance mechanisms, identification of best possible extraction solvents, and improvement of standardized clinical protocols to transform this herbal remedies into practical veterinary application.

In summary, *Curcuma caesia* holds considerable potential as a cost-effective, viable alternative to conventional antibiotics, offering a practical pathway to fight antimicrobial resistance while move forward therapeutic approaches for small animal dermatological care and clinical management.

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