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PLANT-DERIVED ANTIFUNGAL AGENTS IN CANINE OTITIS EXTERNA: EMERGING MOLECULAR PERSPECTIVES AND FUTURE DIRECTIONS

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ABSTRACT
Canine otitis externa (COE) continues to be one of the most common dermatological diseases of companion animals and is facing increasing therapeutic challenges posed by the increasing resistance of antifungal-resistant microbes to existing treatments. Fungicides, especially the azole compounds that are widely used today, are losing efficacy against adaptive resistance mechanisms such as modification of ergosterol synthesis, over-expression of the efflux pumps, and formation of biofilms and their survival. Because of these developments, there is an urgent need to develop new viable and durable drug options. Natural compounds from plants, owing to their myriad phytochemicals and multiple mechanisms, offer some potential therapeutic alternatives that could circumvent the flaws in the contemporary fungicide regimens. Herein, the therapeutic value of Coleus amboinicus and Kalanchoe pinnata is assessed, and C. Amboinicus and K. Pinnata may represent a natural alternative antifungal resource. Furthermore, existing findings regarding their plant chemical constitutions and antimicrobial activity are evaluated along with potential molecular targets. Current limitations in research are critically analyzed, while the potential of bridging plant-derived natural compounds, their chemistry, and molecular targets by applying new knowledge using multidisciplinary research, spanning plant chemistry, molecular pharmacology, veterinary pathology, and infectious disease. Furthermore, the concept of a One Health-based therapeutic framework needs to consider the use of well-chosen, evidence-based natural alternative therapies to successfully treat fungal-derived COE in our companion animals.

KEYWORDS: Canine otitis externa; antifungal resistance; phytotherapy; Coleus amboinicus; Kalanchoe pinnata; molecular mechanisms; One Health; veterinary medicine

1. INTRODUCTION

Canine otitis externa (COE), a very common skin disease in veterinary medicine, affects an estimated 10-20% of dogs globally (Rosser & Patterson, 2020) and involves inflammation of the external ear canal, which is associated with opportunistic fungal pathogens such as Malassezia pachydermatis and Candida species (Nuttall et al., 2018). Although treatment with antifungals has significantly advanced the clinical management of this condition, recurrent infections and treatment failures are becoming increasingly prevalent as the resistance of these fungi to standard antifungals develops (Guardabassi et al., 2019).

The increase in antifungal resistance exhibited by fungal pathogens poses a challenge to both veterinary and human public health. This reduced susceptibility is due to multiple molecular mechanisms, such as mutations occurring in genes involved in ergosterol biosynthesis, overexpression of multidrug efflux pumps, and increased tolerance to oxidative stress, resulting in a survival advantage for the fungal cell upon drug exposure (Sanglard & Coste, 2016; Nett & Andes, 2016). Through these mechanisms, fungal pathogens are capable of resisting repeated prolonged drug treatments, leading to chronic infections and therapeutic non-response (Sherry et al., 2017).

This has Implications for both veterinary medicine and public health as companion animals are in constant proximity with human beings, facilitating the spread of the resistant organisms from one interface to another: human-animal-environment (Weese, 2015). Therefore, antifungal resistance in companion animals becomes a critical issue under the One Health umbrella for maintaining health at all three interfaces (Loy et al., 2022).

As a result, there has been a renewed focus on using natural products as alternatives to chemical antifungals, because they possess naturally occurring bioactive compounds in abundant, structurally diverse forms that can simultaneously target multiple molecular mechanisms in fungi (Cowan, 1999). Plant-derived compounds differ from many modern synthetic antifungals, which target only one enzymatic factor; they have multiple synergistic actions such as disruption of fungal cell membranes, Inhibition of virulence factors, biofilm prevention, and enhancement of oxidative stress (Tiwari et al., 2020). Medicinal plants, such as Coleus amboinicus (variegated Cuban oregano) and Kalanchoe pinnata, have generated scientific interest due to their ethnomedicinal importance and have demonstrated multiple active phytochemicals with documented antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory effects (Olajuyigbe & Afolayan, 2012; Silva et al., 2017). To date, however, relatively few studies have investigated their potential as antifungals for canine

otitis externa from a mechanistic standpoint. The purpose of this Commentary is to review the available scientific evidence concerning plant-derived antifungal agents as future promising alternative therapeutics for canine otitis externa, identify the research gaps in this field, and suggest potential research directions in the development of scientifically validated botanical antifungal agents.

The Expanding Threat of Antifungal Resistance in Canines' Otitis Externa

The growing prevalence of drug-resistant Malassezia species has become a major impediment in the management of canine otitis externa (COE). Typically found in COE infections, Malassezia pachydermatis has a growing number of isolates demonstrating resistance to azoles such as ketoconazole and itraconazole (Cafarchia et al., 2016), rendering readily available first-line treatments ineffective in managing treatment outcomes. As a result, multidrug resistance, the resistance to multiple antifungal drugs by fungal strains, is increasingly documented, reducing the number of available effective therapies and resulting in recurrent infections (Gaitanis et al., 2018; Bensignor & Guillot, 2016).

Furthermore, the clinical challenge of recurrent COE infections is often exacerbated by the presence of biofilms, which protect sessile fungal cells from drugs and host immune responses(Nett & Andes, 2016). Within biofilms, Malassezia, amongst other species, is demonstrably more tolerant to azoles and various other drugs that are not readily detected in planktonic sensitivity testing (Rajendran et al., 2016). This biofilm-associated drug resistance, together with the adaptive resistance exhibited by fungal pathogens in general, results in chronic infections that require novel treatment strategies beyond current drug therapy (Costa-Orlandi et al., 2017). Managing COE, therefore, is an ongoing therapeutic problem that relies on combating both planktonic and biofilm-associated infections effectively in resistant infections.

Molecular Mechanisms Leading to Fungal Resistance to Antifungal Agents

Several molecular mechanisms are involved in the development of resistance to antifungal drugs. A substantial contribution is the mutations observed in ERG11 genes, coding for lanosterol 14-demethylase, the critical enzyme in the ergosterol biosynthesis pathway. Point mutations in ERG11 reduce the binding affinity of azole antifungals to this essential enzyme, thereby altering their efficacy (Flowers et al., 2015). Ergosterol provides essential structural and fluidization properties to the fungal cell membrane; changes in its synthesis not only make the cell tolerant to antifungals but also affect the viability of the fungus (Sharma et al., 2014). Multidrug resistance in fungal species is frequently mediated by the overexpression of ATP-binding cassette (ABC) transporters, a subclass of efflux pumps that remove antifungal drugs from the fungal cell, lowering the intracellular drug concentration (Sanglard & Coste, 2016).

Factors beyond purely genetic and biochemical resistance mechanisms include fungal biofilm formation, which has been previously highlighted, that provides structural defense mechanisms that limit the penetration of antifungal agents within the biofilm (Nett & Andes, 2016). The fungal pathogens' adaptive responses against antifungal drugs are triggered through modulation of the oxidative stress response during exposure to the drug, thereby alleviating the damage caused by ROS (Wang et al., 2018). Cell wall remodeling is the process by which changes occur in the structure and composition of fungal cell wall thereby conferring a degree of resistance to cell wall-targeting drugs (Baker et al., 2011). Quorum-sensing mechanisms control fungal population-behavior, such as virulence factor production and biofilm formation, aiding survival via collective signaling (Hornby et al., 2001). All of these factors work simultaneously, making the targeting of fungal resistance complex and presenting various target pathways for novel antifungal treatments.

The Advantages of Plant-Derived Multitarget Antifungal Agents

In recent years, plants have increasingly drawn interest for their inherent ability to produce compounds with varied bioactive functionalities, including polyphenols, flavonoids, terpenoids, tannins, alkaloids, and volatile compounds or essential oils that possess broad-spectrum activity via multi-target approaches (Tiwari et al., 2021). For polyphenols and flavonoids, the strong antioxidant and membranolytic properties affect and damage cellular integrity and enzymatic processes necessary for life in the cell (Singh et al., 2023). Other classes of compounds, like terpenoids found in various medicinal herbs, interfere with the biosynthesis of the fungal wall and the mitochondria's functions. Tannins denature proteins. They break the cohesion in the Fungal cell wall and membranes (Kumar et al., 2022). Alkaloids attack cellular DNA synthesis and inhibit the formation of fungal biofilm (Patel & Patel, 2024). Lastly, the mixed-composition volatility or the components known as Essential oils (EOs) exert highly active killing and inhibitory properties often linked with fungal and mitochondrial (membrane) damage as well as generation of reactive oxidative species (ROS) (Liu et al., 2020). The presence of several active phytochemical constituents in extracts would result in a plant-derived multitarget antifungal approach, contrary to the classic synthetic antifungal compounds designed to target single steps in fungal enzymes like ergosterol synthesis (Sharma & Gupta, 2022). The broad efficacy observed across multiple targets would minimize chances for acquisition of drug resistance, since the probability of mutation in several independent pathways simultaneously is unlikely (Dias et al., 2023). For instance, compounds interacting with membranes, preventing biofilm formation and ROS generation, create an extreme stress environment on fungi and the resulting limited options to develop survival strategies. (Narayanan et al., 2021). Also, the combination of a few compounds will produce a positive interaction of synergism, improve efficacy, and decrease minimal inhibitory concentrations, reducing the pressure for resistance. Furthermore, most plant-derived products have less toxicity, thus making it a safe alternative to synthetic counterparts and fitting in contemporary natural approach in veterinary and even human therapy (Zhou et al., 2025). The immunostimulatory and anti-inflammatory potential of plant derivatives makes these approaches a useful aid in therapeutic strategy, in cases such as canine otitis externa fungal infections. Since new compound development is crucial because of the high prevalence of azole and fluoroquinolone-resistant fungal infections, exploring multitarget plant derivatives offers an efficient potential solution (Singh & Kaur, 2023).

Coleus amboinicus: Emerging Molecular Evidence

The scientific community has had a particular interest in this species due to the high availability of active phytochemical constituents like carvacrol, thymol, rosmarinic acid, and varied types of flavonoids in its tissues (Lima et al., 2023). Carvacrol and thymol, monoterpenic phenols, have a remarkable antimicrobial activity mainly due to their action on cell membrane levels (Naeini et al., 2022). Rosmarinic acid, a phenolic compound, displays strong antioxidant activity that induces damaging generation of ROS (Zhao et al., 2021), while flavonoids interfere with key cellular processes and enzymatic targets in fungi (Patel et al., 2024).

The compounds identified in Coleus amboinicus exert their fungicidal activity in several molecular ways. One is via disruption of fungal cell membranes when carvacrol and thymol insert into lipid bilayers and destabilize their structure (Kumar et al., 2022). Secondly, they hinder and interfere with ergosterol synthesis, which is essential for stabilizing fungal cell membranes and creating weak spots in them (Singh & Sharma, 2023). The induction of intracellular ROS generation caused by plant compounds also plays a crucial role in inducing cell death in fungal colonies (Zhao et al., 2021). In addition to inhibiting direct fungicidal effects, Coleus amboinicus compounds are capable to inhibit fungal adhesion to surfaces of biological tissues as well as formation of biofilm (Lima et al., 2023), thereby preventing invasion and survival.

Kalanchoe pinnata: Beyond Traditional Medicine

This plant used broadly in traditional medicine possess interesting profile phytochemicals such as bufadienolides (class of cardiac glycosides with known anti-microbial activity (Singh et al., 2022)) Flavonoids (quercetin and kaempferol exhibit antimicrobial activity through their capacity to interfere in different targets as well as their antioxidant (Liu et al., 2024)) and Phenolic acids that can disrupt some cellular pathways or modify the generation of oxidative reactive species in fungal strains (Patel & Kumar, 2023). The mechanisms of action of Kalanchoe pinnata constituents towards this class of microorganism are mainly via the destabilization of Fungal membrane caused by bufadienolides and some flavonoids that facilitate the efflux of its contents (Owolabi et al., 2023). Also, the action upon mitochondrial members, which led to impairment of energy supply and apoptosis-like cell death (Singh et al., 2022), was shown, in addition to enzyme inhibition by quercetin and kaempferol. The plant also shows an interesting antioxidant property, enabling modification of oxidative response in Fungi. (Liu et al., 2024). The multitarget effects described for this compound offer great possibilities as an alternative in controlling chronic persistent fungal Infections like that seen in Canine Otitis Externa: Aetiology.

Future Molecular Research Directions

The continued investigation and understanding of the mechanisms that determine azole resistance in canine otitis externa includes the combined efforts from basic scientists and practitioners of Veterinary Medicine using an integrated approach, One Health strategy. Molecular Biology will need to focus on whole- genome sequencing (WGS) of selected-resistant fungal strains to establish the basis for this resistance. Other modern multi-omic tools, such as comparative transcriptomics, proteomics, and metabonomics, will help clarify how such genes are regulated in resistant strains. Expression levels of specific targets during exposure to antifungal compounds can illuminate the molecular pathways implicated. Furthermore, application of CRISPR tools may allow the identification of causal resistance-associated genes/pathways and assist in functional analyses (Tiwari et al., 2021; Singh et al., 2023).

Phytochemistry will further characterize the structures of bioactive secondary metabolites present in different plant extracts, using techniques such as LC-MS / MS,GC- MS, fingerprints by HPLC and structural determination using NMR in plant extracts such as Coleus amboinicus and Kalanchoe pinnata, allowing more standardization and identification of efficient drugs. (Lima et al., 2023; Owolabi et al., 2023).

Mechanistic studies will employ computational and systems biology methods to improve understanding of drug mechanisms of action and resistance. Using tools like docking and molecular dynamics simulation to analyze the interaction between phytochemicals and fungal targets is important. Integration of data from multiple 'omics fields into systems biology platforms will help the construction of molecular interaction networks between drugs and fungal targets and will provide leads to the development of multiple anti-fungal approaches. (Kumar et al., 2022; Sharma & Gupta, 2022).

Veterinary Medicine will focus on the safety and clinical efficacy of plant-based compounds via well-designed clinical trials for dogs. This research must examine different dosing protocols and routes of administration, and formulation. Combinatory use of plant compounds alone and with proven antifungals (like azoles) to treat clinical canine cases should be explored. (Zhou et al., 2025). Surveillance of antifungal resistance in companion animals should continue as part of ongoing surveillance programs in other animal and human sectors (a One Health approach) to allow the tracking of trends and anticipate threats. Research efforts focused on companion animal microbiomes and environmental contamination will provide a better understanding of the pathways of transmission. Best practices and stewardship across veterinary and human medicine, both within and between countries, will minimize selection for antifungal resistance and thus maintain our capacity to treat resistant fungal infections and preserve human health.

CONCLUSION

The increasing problem of antifungal resistance during canine otitis externa requires a holistic, multidisciplinary approach at the intersection of molecular biology and translational science. The application of the state of the art molecular biology methods, like whole-genome sequencing and CRISPR functional studies, will enable uncovering the molecular and regulatory basis of resistance and, accordingly, allow a directed therapy targeting the underlying causes. A detailed

phytochemical characterization of lead candidates will deliver standardizable bioactive compounds with multitarget antifungal activity, possibly at the same time, by overcoming some drawbacks of synthesized drugs. Clinical validation by well-designed trials in the veterinary context, together with pharmacokinetic and toxicological data, will translate the generated information safely and effectively in practice. Most importantly, the application of the one health concept through monitoring, microbiome studies, and responsible administration will allow stopping the spread of resistant fungi from animals to humans and the environment. Therefore, we hope that by implementing this research program, the management of animal fungal infections can be improved by sustainable treatments and maintain their effectiveness in resistance situations.

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