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Homeopathic potential of medicinal plants in fighting microbial overgrowth found in canine otitis

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Abstract

Canine otitis, commonly known as a canine ear infection, is a frequent issue in veterinary medicine. The condition is typically caused by bacterial or fungal imbalances within the microbiome of the ears. Although antimicrobial treatments for these overgrowths are available, a veterinary visit is required, treatment can be expensive, and antibiotic resistance is a potential concern. In fact, during a recent survey, 48% of owners stated that they would be willing to attempt an at-home, natural remedy using easily-sourced ingredients before visiting their local veterinary office. The current project aimed to build on existing research to determine the viability of at-home treatment options for canine otitis, using supplies and methods easily accessible and reproducible for the average pet owner. Several common plants with known medicinal properties were used to create crude extracts and infused oils using household supplies. These extracts were then applied to pure cultures of *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* (two of the most common microorganisms to cause infection) to determine whether each extract inhibited pathogen growth and to allow for comparison between the different extract preparation techniques. It was anticipated that the crude extracts would have a more significant inhibitory effect than the infused oils but that both had the potential to suppress microbial growth. Of all the medicinal plants tested, clove was the most successful at suppressing the growth of *S. pseudintermedius* but had no effect on *M. pachydermatis*. Meanwhile, when garlic was extracted at 36 °C for a 24 hr period, it showed minor inhibitive properties when tested on both microbes. Future studies may include varying the concentrations of the extracts used and comparison between inhibitory zones of extracts against routine prescriptive medicine.

Homeopathic potential of medicinal plants in fighting microbial overgrowth found in canine otitis

Canine otitis, commonly known as a canine ear infection, is a common issue in small animal veterinary medicine, with more than 46% of dog owners reporting a history of canine otitis. A comprehensive study of the microbiome of dogs with ear and skin infections found that bacterial and fungal overgrowth was the most common cause of infection. Specifically, the most prevalent bacteria in infected ears were *Staphylococcus* subspecies, such as *S. pseudintermedius* and *S. schleiferi*, and gram-negative *Pseudomonas aeruginosa*. The most common fungal pathogen was *Malassezia pachydermatis* (Tang et al., 2020).

Although antimicrobial treatments for these overgrowths are available, those options require a time-consuming veterinary exam and prescription purchase, which can be expensive. In addition, some of these common bacterial and fungal species have developed a resistance to the primary treatment and may require multiple courses of medication to resolve an infection. Compounding this issue, some dog owners would prefer to try a natural treatment before resorting to antimicrobial prescriptions to help combat antibiotic resistance and avoid subjecting their pets to substances that may have concerning side effects. This was confirmed during a recent survey where approximately 48% of individuals reported being interested in trying at-home remedies to treat their pet's ailments. At-home treatments may involve purchasing premade natural tinctures or creating natural concoctions from scratch, often using essential oils.

Plants have been used for hundreds of years in homeopathic remedies, and many published studies have examined the effectiveness of essential oils from known medicinal plants against various pathogens. For example, one recent study determined that a diluted clove essential oil spray successfully reduced the number of the viable specimens of *M. pachydermatis* and *S. pseudintermedius* in isolated cultures by 99.9% (Aiemsaard et al., 2020). Another examined the effectiveness of 22 different essential oils, including clove, lemongrass, cinnamon leaf, oregano, and tea tree oil, against *M. pachydermatis*. While effectiveness varied by essential oil, overall, the study showed essential oils hold promise in treating fungal infections (Bismarck et al., 2020). Similarly, oregano and garlic essential oils have demonstrated inhibitory effects on *P. aeruginosa*, *S. aureus*, and *E. coli* (Bouhdid et al., 2009; Wolde et al., 2018). An all-natural topical skin cream, consisting of tea tree oil, avocado oil, emu oil, and jojoba oil, was also found to halt the growth of specific pathogenic bacterial and fungal strains after 12 h of incubation, using in vitro testing methods (Han et al., 2016). Unfortunately, while all of these studies show promise for the use of essential oils as an alternative to the current antimicrobial medications available; few studies use methodology readily reproducible by the average pet owner, a focus of the current project.

Working from the existing research, this project aimed to determine the viability of at-home treatments for canine otitis using supplies and methods easily accessible and reproducible for the average person, using homegrown herbs and or their derived oils, or plants purchased from local or online businesses.

A list of common plants with known medicinal properties was compiled, and 6 were chosen for experimentation: clove, oregano, garlic, calendula, cinnamon, and green tea. Oregano and garlic, in particular, were purchased fresh or grown at home before harvesting and dehydrating. Fresh herbs were dehydrated to ensure freshness and act as a control. The other medicinal plants were obtained, in their dried form, through online purchase. All tissues were ground and prepped for water and oil extraction methods.

Water extractions were created by heating ground plant tissues in water at 36 °C for 24 hr. Alternatively, they were also heated at 60-65 °C for 20, 40, and 60 min and then stored for 12-24 hr at room temperature. Once complete, all extracts were refrigerated until use. The oil extraction methodology was modified from a similar technique reported online (Irene). Here, dried plant parts were ground and infused in vodka for 24 hr, then blended in oil for 5 min, strained, and stored in an airtight container in a cool, dark place.

Each extract was tested by pipetting volumes of 10, 20, and 30 μL onto petri dishes with isolated cultures of *M. pachydermatis* and *S. pseudintermedius* and incubated for 24-72 hr before being visually examined for their inhibitory properties. A solution of a commonly prescribed antimicrobial, Baytril® Otic, was used as a positive control (Baytril® Otic Antibacterial-Antimycotic Emulsion | For Veterinary Professionals). While this method could not determine whether the extracts were microbicidal or microbistatic, it could indicate if an extract had inhibitory properties by showing an absence of growth where the extract was applied. This method also allowed for comparison between different preparation

techniques (e.g., various essential oils, brewing temperatures, and infusion methods).

Extracts had the most informative effect on isolated cultures of *S. pseudintermedius*, but testing was not without complications. Clove extract had the most significant inhibitory effect on *S. pseudintermedius,* producing an inhibitory zone with water extraction techniques. Garlic was the next most successful extract, but not all extraction methods produced inhibitory effects. Only the extract heated to 36 °C for 24 hr produced a response that resulted in a minor inhibitor zone. Several plates yielded inconclusive results due to inconsistencies in treatment areas, with extracts failing to absorb properly into the agar and traveling toward different plate sectors or unfiltered plant debris being pipetted onto the plate along with the liquid extract itself. Both circumstances made visual determination of inhibitory zones too challenging to establish. Outside of clove and garlic (which was inconclusive), none of the extracts heated for the 20 or 40 min intervals at 60-65 °C showed any effectiveness.

Treatment of isolated cultures of *M. pachydermatis* resulted in similar challenges to that of *S. pseudintermedius,* with the extract droplets running across the plate. However, the more significant issue in this group was microbial contamination on several plates, which made interpretation difficult and caused doubt regarding treatment success. Despite these concerns, garlic extraction at

36 °C for 24 hr showed clear inhibitive potential, consistent with results seen when applied to *S. pseudintermedius.* Additionally, while the results were not as pronounced, oregano also indicated some inhibitive properties when extracted at 36 °C for a 24 hr. Regrettably, the oleophobic properties of the agar were not considered when testing oil extracts, which had a significant impact that resulted in inconsistent treatment areas, making interpretation unclear.

Overall, this project produced interesting preliminary data that should be followed by a more substantial study, revising the mythology to avoid confounding issues seen here. Future studies should consider using filtered pipette tips to eliminate plant particulates when plating, finding an alternative testing medium for the oil extracts, and increasing replication to determine how reliable initial results may be. Additionally, because extract volume did not seem to impact the extracts' effectiveness, an alternate variable might be to create extracts of varying concentrations to determine if the concentration might impact observed results. After collecting more information on the efficacy of these prepared extracts (using a revised methodology), effectiveness should be compared to frontline prescription treatments.

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