RESEARCH ARTICLE

Therapeutic Efficacy of *Allium sativum* and *Calendula officinalis* against Secondary Otitis in Dogs

Srashty Singh[1], Amita Tiwari[1], Devendra Kumar Gupta[1], Ashutosh Mishra[2], Pooja Dawar[1]

Abstract

Secondary otitis is a common inflammatory ear condition in dogs associated with bacterial and fungal infections. The increasing concern regarding antimicrobial resistance has encouraged the exploration of herbal alternatives with antimicrobial and anti-inflammatory properties. The present study was undertaken to evaluate the therapeutic efficacy of *Allium sativum* and *Calendula officinalis* infused oils against secondary otitis in dogs. Eighteen dogs with cytologically confirmed secondary otitis were randomly divided into three treatment groups (n=6 each). Six apparently healthy dogs were kept as the control group. Group G1 received *Calendula officinalis* infused oil, Group G2 received *Allium sativum* infused oil, and Group G3 received a combination of both infused oils topically 4-5 drops twice daily for 14 days. Clinical index score, yeast count, and bacterial load were evaluated on days 0, 7, and 14. Significant improvement in clinical and cytological parameters was observed in all treatment groups. The combination therapy (G3) showed the highest therapeutic efficacy with 92.11 % reduction in clinical score and 87.22 % reduction in yeast count by day 14 post-treatment, along with restoration of bacterial count to normal levels in all treated dogs. Group G1 showed better response than Group G2. The findings indicated that combined topical administration of both infused oils is an effective herbal therapy for secondary otitis in dogs.

Key words: *Allium sativum*, *Calendula officinalis*, Cytology, Dogs, Secondary otitis.

Ind J Vet Sci and Biotech (2026): 10.48165/ijvsbt.2026.22.06.22

Introduction

Otitis signifying unilateral or bilateral ear inflammation, targets the external auditory canal (otitis externa), epithelial lining or structures in the middle ear (otitis media), or the inner ear (otitis interna). Prevalence of the most common ear infection otitis externa, 5-20 % globally, is notably higher (30-40 %) in the tropical environment (Fernandez *et al*., 2006). Infectious otitis externa is clinically manifested as a secondary complication of factors that induce inflammation within the ears. These include atopic dermatitis, deleterious cutaneous reactions to food, foreign bodies, ectoparasites, metabolic diseases, keratinization anomalies, and autoimmune diseases. The predisposing factors in otitis externa comprise long pendulous ear pinna, stenotic ear canal, moisture retention, excessive hair in the external ear canal leading to heat retention, and inappropriate treatment (Rosser, 2004). A plethora of pathogenic bacteria: *Staphylococcus pseudointermedius*, *Pseudomonas aeruginosa, Escherichia coli, Proteus*, *Corynebacterium*, *Klebsiella spp.* and the fungal agent, *Malassezia pachydermatis* are the major aetiological agents (Zur *et al*., 2011).

In otitis externa, the inflammatory process promotes erythema of the pinnae, external meatus and lining of the external canal of varying magnitude. Subsequently, a wide range of clinical signs, namely head shaking, ear scratching, ceruminous or purulent otic discharge, self-trauma and aural hematomas and acute moist dermatitis near the base of ear, foul odour, swelling, and pain are observed (Rosser, 2004). Diagnostic protocol involves identification of the bacterial and fungal isolates, based on the cultural, morphological and biochemical parameters (Engelen *et al.,* 2010). Aseptically collected samples from the external auditory canal are heat fixed and stained with methylene blue or Diff-Quik stain (Saridomichelakis *et al.,* 2007).

Topical therapy is the benchmark of successful resolution of otitis externa, surface infection. Medical therapy involves cleaning of the external ear canal and local therapy with antimycotic agents, antibiotics, corticosteroids and other topical agents (Morris, 2004). Currently, antibiotic resistance is increasing exponentially, posing a formidable universal

[1]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur-482001 (MP), India

[2]Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur-482001 (MP), India

Corresponding Author: Dr. Srashty Singh, P.G. Scholar, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur-482001 (MP), India. e-mail: singh.srashty@gmail.com

How to cite this article: Singh, S., Tiwari, A., Kumar Gupta, D., Mishra, A., & Dawar, P. (2026). Therapeutic Efficacy of *Allium sativum* and *Calendula officinalis* against Secondary Otitis in Dogs. Ind J Vet Sci and Biotech, 22(6), 130-134.

Source of support: No specific funding was received for this study.

Conflict of interest: None

Submitted 24/07/2026 **Accepted** 15/09/2026 **Published** 10/11/2026

© The Author(s). 2026 Open Access This work is licensed under a Creative Commons Attribution-Non Commercial-No Derivatives 4.0 International License.

health issue. In the biomedical scenario, it is imperative to search for potent alternatives to antibiotics. *Allium sativum* (family Liliaceae), popularly known as clove garlic, is one of the nature's most versatile medicinal plants. In addition to high demand as a healthy food ingredient, garlic is endowed with antiviral, antibacterial, antifungal, antioxidant, antiatherosclerotic and anticancer properties (Wilson *et al.,* 2019). *Calendula officinalis* (family Asteraceae), widely used in homeopathic medicine in the treatment of many human diseases, is reported to possess a wide range of pharmacological activity: antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral. This medicinal plant is also endowed with cytotoxic and tumour-suppressing propensity (Ramadhan *et al.,* 2015). This study was planned to evaluate the therapeutic efficacy of *Allium sativum* and *Calendula officinalis* against secondary otitis in dogs

Materials and Methods

The present study was conducted at Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur (India) for a period of 6 months (May-October, 2022) on dogs presented with signs of otitis. All the animals were examined for the presence of clinical symptoms like local pain perception, head tilt to one side, pruritus, erythema, ear discharge, malodour, crusting and desquamation of the epithelium etc. Dogs demonstrating clinical signs pertaining to secondary otitis were subjected to cytological examination for confirmation, amongst which 18 positive cases were selected for the experiment.

Clinical Evaluation

A clinical index score (CIS) was assigned to all dogs in the treatment groups before treatment (day 0) and after treatment (day 14). Ear inflammation was evaluated using four parameters - erythema, exudate, pain/discomfort, and swelling - each scored on a scale of 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). The maximum possible CIS was 12. Dogs with a CIS of 6 or higher were included in the therapeutic study (Engelen *et al.,* 2010).

Collection of Samples and Cytological Examination

Samples of ear exudate were collected from the horizontal portion of the external auditory canal with the help of a sterile swab moistened with sterile iso-osmotic saline solution (Kiss *et al.,* 1997). From each ear two swabs were carefully collected and suitably labeled. The samples were collected on day 0 (pre-treatment) and also on day 7 and day 14 post-treatment.

Smears were prepared from the individual samples collected from the inflamed ear canals by rolling of cotton swabs onto clean, dry glass slides. The slides were completely air dried, briefly heat-fixed and stained with new methylene blue. The stained slides were examined under high power oil immersion lens (x1000) for morphological identification and counting of microorganisms. The results were considered abnormal if at least one of the following stipulated conditions was noted, yeast spp ≥ 10/HP field; bacteria ≥ 5/HP field. The significance of bacterial load in cytological smear was evaluated on the basis of criteria recommended as normal ≤ 4 bacteria; gray zone 5-24 bacteria, & abnormal if ≥ 25 bacteria per high power field (Angus, 2004).

Collection and Processing of Plants/Oils

Fresh flowers of *Calendula officinalis* and fresh cloves of *Allium sativum* were obtained from the Departments of Horticulture and Plant Physiology, respectively, at the Agriculture College, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (India). The plant materials were manually cleaned to eliminate extraneous matter and initially shade-dried in a clean, well-aerated environment. Final drying was carried out in an incubator maintained at 40°C to ensure complete removal of moisture. The dried samples were then pulverized using an electric grinder to obtain a coarse powder, which was stored in airtight containers with appropriate labeling for further preparation of infused oils.

Dried *Calendula officinalis* powder, dried *Allium sativum* powder were each infused in extra-virgin olive oil at a ratio of 1:10 (w/w), while the combined *Calendula officinalis* and *Allium sativum* preparation was infused at a ratio of 1:5 (w/w) each. All mixtures were maintained in the dark for 21 days, after which the infused oils were filtered through Whatman filter paper No. 1 using a separating funnel. The filtrates were stored in airtight containers at 4 °C in the dark until further experimental use (Gumus *et al.,* 2015).

Experimental Design

Total 18 dogs, positive for secondary otitis, were randomly distributed in three treatment groups: G_1, G_2 and G_3 each comprising six animals and were administered infused *Calendula officinalis* oil, *Allium sativum* oil, and a combined *Allium sativum* and *Calendula officinalis* oil preparations @ 4-5 drops, twice daily topically, for 14 days. Need-based supportive treatment was also given as per the standard doses. Six apparently healthy dogs were kept as the control group, G_4.

Therapeutic Efficacy Evaluation

The comparative efficacy of the three treatment regimens was assessed using clinical recovery and cytological findings evaluated by mean percentage reduction in clinical score and yeast count on days 7 and 14 post-treatment. A reduction

Table 1: Clinical index score in dogs in different treatment groups at different intervals (Mean, Median and Range data)

Criteria	Groups (n=6)	Day 0	Day 7	Day 14	Mean % reduction		χ^2 value (P value)
					Day 7	Day 14	
Clinical index score	G_1	7.83, 7.50^A (6, 10)	4.00, 4.00^B (2, 6)	0.83, 0.50^C (1, 2)	48.91	89.40	12.60 (0.002)**
	G_2	7.17, 7.0^A (6, 9)	4.00, 4.00^B (3, 5)	1.00, 1.00^C (0, 2)	44.21	85.91	12.60 (0.002)**
	G_3	8.50, 8.50^A (6, 11)	3.83, 4.00^B (2, 5)	0.50, 0.00^C (0, 2)	54.94	92.11	12.60 (0.002)**
	P value	0.399^{NS}	0.983^{NS}	0.875^{NS}		–	
Yeast count	G_1	14.17, 14.00^A (11, 18)	7.17, 7.00^B (5, 10)	2.33, 2.50^C (0, 4)	49.40	83.55	12.60 (0.002)**
	G_2	13.83, 13.50^A (10, 18)	7.67, 7.50^B (6, 10)	2.50, 3.00^C (0, 4)	44.54	81.92	12.60 (0.002)**
	G_3	14.67, 14.00^A (11, 19)	6.50, 6.50^B (5, 8)	2.17, 2.00^C (0, 5)	55.69	87.22	12.60 (0.002)**
	P value	0.864^{NS}	0.491^{NS}	0.795^{NS}		–	

Mean values with different superscripts between days (uppercase) differ significantly (p<0.01); **P<0.01, NS- non-significant (p>0.05).

exceeding 50% in clinical score and mean yeast count, along with restoration of bacterial counts to normal levels, was considered indicative of a positive therapeutic response

Statistical Analysis

The data pertaining to the mean per cent reduction in clinical index score and yeast count were analyzed by using Kruskal-Walli's ANOVA between groups. Similarly, between intervals comparison for each group was done using Mood's Median test followed by Wilcoxon signed-rank test for pair-wise comparison (Snedecor and Cochran, 1994).

Results and Discussion

The response to therapy in different groups was determined on the basis of per cent reduction in yeast count and clinical score, *viz.,* erythema, pain, swelling, exudate and restoration of bacterial count to normal on day 7 and day 14 post-treatment. Dogs of group G_3 showed 92.11 % and 87.22 % reduction in clinical score and yeast count, respectively, and bacterial count was restored to normal in all the 6 dogs on day 14 post-treatment. Similarly, in group G_1 reduction in clinical score and yeast count was 89.40 % and 83.55 %, respectively, with restoration of bacterial count to normal in 4 out of 6 dogs. Whereas, on day 14 post-treatment, group G_2 showed a reduction of 85.91 % in clinical score, 81.92 % in yeast count, and restoration of bacterial count to normal in 3 out of 6 dogs. Therefore, on the basis of clinical recovery and restoration of cytological count to normal, the most effective response for secondary otitis was elicited by the group administered with combination of *Allium sativum* and *Calendula officinalis* infused oil (G_3)(Table 1, 2).

Comparative Therapeutic Efficacy

The efficacy of drugs used was assessed based upon clinical index score and cytological evaluation. Clinical index score (CIS) and yeast count values between 0^{th}, 7^{th} and 14^{th} day were found to be significant in all the groups, *i.e.,* G_1-G_3. Pair-wise comparison revealed that there was significant decrease in CIS values between 0^{th} and 7^{th} day in all the groups. Similarly, there was also significant decrease in the CIS values between 7^{th} and 14^{th} day and 0^{th} and 14^{th} day in all the treatment groups (Table 1). Bacterial count on day 0 was found to be abnormal. However, periodical checking of samples on day 7^{th} and 14^{th} revealed bacterial count to be drastically reduced in all the groups (Table 2).

Table 2: Bacterial count in dogs in different treatment groups at different intervals

Groups (n=6)	Criteria	Bacterial count		
		Day 0	Day 7	Day 14
G_1	Abnormal	6	2	0
	Gray zone	0	3	2
	Normal	0	1	4
G_2	Abnormal	6	3	1
	Gray zone	0	3	2
	Normal	0	0	3
G_3	Abnormal	6	0	0
	Gray zone	0	4	0
	Normal	0	2	6

In treatment group G_3, on day 14 post-treatment mean per cent reduction in clinical index score and yeast count was 92.11 and 87.22, respectively and bacterial count was restored to normal (≤ 4 bacteria/OIF) in all the 6 dogs proving combination of *Allium sativum* and *Calendula officinalis* infused oil as the best followed by group G_1 and G_2. The bacterial count in dogs with secondary otitis was categorized as abnormal, gray zone and normal at the day of presentation. Bacterial count was found to be abnormal in all the six dogs on day 0 in all 3 groups. However, upon initiation of therapy the bacterial count in G_1 was abnormal in 2 dogs, under gray zone in 3 dogs and normal in 1 dog on day 7, whereas on day 14 (post-treatment) bacterial count in 2 dogs was under gray zone and became normal in 4 dogs. In group G_2, the bacterial count in abnormal, gray zone and normal category was found in 3, 3, and 0 dogs on day 7, and

1, 2 and 3 on day 14, while in G_3 baxcterial count came to normal in all 6 dogs by day 14 post-treatment (Table 2).

Calendula officinalis possesses various biological active constituents such as carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids etc. (Kishimoto *et al.,* 2005; Roopashree *et al*., 2008). The extracts exert a wide range of pharmacological effects and are used as antiseptic, analgesic, antiviral, antifungal, antibacterial and wound healing agents (Preethi *et al.,* 2006). *Calendula* has various antifungal and antibacterial activities and has been used for the treatment of abrasions, burns, ulcers, skin inflammation, eczema and wounds (Schulz *et al.,* 2004). Volatile oils such as t-rt and palustron present in *Calendula* flowers are known to show potent antimicrobial activity *in-vitro* against various fungal strains, gram-positive and gram-negative bacteria (Jan *et al.,* 2017). Terpenoids (calendulaglycosides A and B) and flavonoids such as quercitin, isoquercitin, rutin etc. of *Calendula* flowers show anti-inflammatory activity and antioxidant properties. *Calendula* facilitates the wound healing by increasing wound angiogenesis, epithelialization and collagen metabolism leading to improvement in local circulation and granulation tissue formation (Leach, 2008).

Allium sativum exhibhits antibacterial, antifungal, antioxidant, immunomodulatory, antiseptic and anticancerous properties (Li *et al.,* 2015; Saha *et al.,* 2016; Wilson *et al.,* 2019). Allicin a notable flavonoid in garlic is the most predominant thiosulphate that is responsible for its potent antibacterial, antifungal properties against Gram-positive, Gram-negative bacteria and yeast including *Candida* spp. (Okere *et al.,* 2014; Uzun *et al.,* 2019). The organosulfur compounds derived from garlic such as allin, allicin and diallyl sulfide are known to possess antioxidant properties (Tapiero *et al.,* 2004). Meanwhile, the antimicrobial potency of garlic could be attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis.

Combination of these two indigenous herbs offered better reduction in the count of secondary organisms in the affected ears, along with greater improvement in the clinical profile of the patients, compared to when given alone. Perhaps, concurrent administration of '*Calendula officinalis*' and '*Allium sativum*' infused oil topically in affected ears of dogs elicited a noteworthy synergistic bio-response, vis-à-vis antibacterial, antifungal, and anti-inflammatory effect coupled with better antioxidative effect of extra virgin olive oil.

Conclusion

The study indicated that topical application of *Allium sativum* and *Calendula officinalis* powder infused in olive oils produced appreciable clinical and cytological improvement in dogs affected with secondary otitis. The combined herbal formulation yielded better therapeutic response than the individual treatments, as evidenced by greater reduction in clinical score, yeast count, and bacterial load. The enhanced efficacy may be associated with the antimicrobial, antifungal, anti-inflammatory, and antioxidant activities of the phytoconstituents present in both medicinal plants. These findings highlight the potential utility of the combined herbal preparation as an alternative therapeutic approach for canine secondary otitis. Further investigations using larger sample populations and advanced microbiological studies are necessary to substantiate its clinical usefulness.

Acknowledgement

The authors are thankful to the Department of Veterinary Medicine, College of Veterinary Science and AH, NDVSU, Jabalpur, for providing necessary facilities to carry out the present study.

References

Angus, J. C. (2004). Otic cytology in health and disease. *Veterinary Clinics of North America: Small Animal Practice, 34*, 411–424.

Engelen, M., De Bock, M., Hare, J., & Goossens, L. (2010). Effectiveness of an otic product containing miconazole, polymyxin B and prednisolone in the treatment of canine otitis externa: Multisite field trial in US and Canada. *International Journal of Applied Research and Veterinary Medicine, 8*(1), 21–30.

Fernandez, G., Barboza, G., Villalobos, A., Parra, O., Finol, G., & Ramirez, R. A. (2006). Isolation and identification of microorganisms present in 53 dogs suffering otitis externa. *Scientific Journal—University of Zulia Faculty of Veterinary Sciences Research Division, 16*(1), 23–30.

Gumus, Z. P., Guler, E., Demir, B., Barlas, F. B., Yavuz, M., Colpankan, D., & Timur, S. (2015). Herbal infusions of black seed and wheat germ oil: Their chemical profiles, *in vitro* bio-investigations and effective formulations as phyto-nanoemulsions. *Colloids and Surfaces B: Biointerfaces, 133*, 73–80.

Jan, N., Andrabi, K. I., & John, R. (2017). *Calendula officinalis*—An important medicinal plant with potential biological properties. *Indian National Science Academy, 83*(4), 769–787.

Kishimoto, S., Maoka, T., Sumitomo, K., & Ohmiya, A. (2005). Analysis of carotenoid composition in petals of *Calendula officinalis* L. *Bioscience, Biotechnology, and Biochemistry, 69*(11), 2122–2128.

Kiss, G., Radvanyi, S., & Szigetti, G. (1997). New combination for the therapy of canine otitis externa—Microbiology of otitis externa. *Journal of Small Animal Practice, 38*, 57–60.

Leach, M. J. (2008). *Calendula officinalis* and wound healing: A systematic review. *Wounds, 20*(8), 236–243.

Li, G., Ma, X., Deng, L., Zhao, X., Weil, Y., Gao, Z., Jia, J., Xu, J., & Sun, C. (2015). Fresh garlic extract enhances the antimicrobial activities of antibiotics on resistant strains *in vitro*. *Jundishapur Journal of Microbiology, 8*(5), 1–6.

Morris, D. O. (2004). Medical therapy of otitis externa and otitis media. *Veterinary Clinics of North America: Small Animal Practice, 34*, 541–555.

Okere, O. S., Sangodele, J. O., Adams, M. D., Ogunwole, E., & Shafe, M. O. (2014). Antibacterial and antifungal activity of methanolic leaf extract of *Allium sativum* on selected pathogenic strains. *International Journal of Tropical Disease and Health, 4*, 1146–1152.

Preethi, K. C., Kuttan, G., & Kuttan, R. (2006). Antioxidant potential of an extract of *Calendula officinalis* flowers *in vitro* and *in vivo*. *Pharmaceutical Biology, 44*(9), 691–697.

Ramadhan, R. S., Hussein, Z. F., & Dulaimi, R. K. (2015). Antibacterial and anti-inflammatory activities of *Calendula officinalis* growing in Iraq. *World Journal of Pharmacology and Research, 4*(4), 25–33.

Roopashree, T. S., Dang, R., Rani, R. H., & Narendra, C. (2008). Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis*. *International Journal of Applied Research in Natural Products, 1*(3), 20–28.

Rosser, E. J. (2004). Causes of otitis externa. *Veterinary Clinics of North America: Small Animal Practice, 34*(2), 459–468.

Saha, S., Saha, S. K., Hossain, M. A., Paul, S. K., Gomes, R. R., Imtiaz, M., Islam, M. M., Nahar, H., Begum, S. A., & Mirza, T. T. (2016). Anti-bacterial effect of aqueous garlic extract (AGE) determined by disc diffusion method against *Escherichia coli*. *Mymensingh Medical Journal, 25*, 23–26.

Saridomichelakis, M. N., Farmaki, R., Leontides, L. S., & Koutinas, A. F. (2007). Aetiology of canine otitis externa: A retrospective study of 100 cases. *Veterinary Dermatology, 18*(5), 341–347.

Schulz, V., Hansel, R., Blumenthal, M., & Tyler, V. E. (2004). *Rational phytotherapy: A reference guide for physicians and pharmacists* (5th ed.). Springer.

Snedecor, G. W., & Cochran, W. G. (1994). *Statistical methods* (8th ed.). Iowa State University Press.

Tapiero, H., Townsend, D. M., & Tew, K. D. (2004). Organosulfur compounds from Alliaceae in the prevention of human pathologies. *Biomedicine & Pharmacotherapy, 58*(3), 183–193.

Uzun, L., Dal, T., Kalcıoglu, M. T., Yurek, M., Acikgoz, Z. C., & Durmaz, R. (2019). Antimicrobial activity of garlic derivatives on common causative microorganisms of the external ear canal and chronic middle ear infections. *Turkish Archives of Otorhinolaryngology, 57*(4), 161–165.

Wilson, A., Pandya, D., Mankad, A., & Solanki, H. (2019). A review on *Allium sativum* (L.) as medicinal plant. *International Journal of Research and Analytical Reviews, 6*(1), 141–147.

Zur, G., Lifshitz, B., & Bdolah-Abram, T. (2011). The association between the signalment, common causes of canine otitis externa and pathogens. *Journal of Small Animal Practice, 52*, 254–258.