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Antibacterial Activity of Traditional Herbal Extracts Against *corynebacterium pseudotuberculosis*

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ABSTRACT

Corynebacterium pseudotuberculosis, which causes caseous lymphadenitis (CLA) in sheep and goats, represents a major threat to Pakistan's livestock industry. The chronic infection known as CLA results in the formation of abscesses that affect both lymph nodes and internal organs, which causes substantial economic damage. The rising rates of antimicrobial resistance (AMR) today result from extensive antibiotic use, which has created a need for alternative therapies that originate from natural sources. The research examines the antibacterial properties of Fagonia cretica (Dhamasha Booti), Withania coagulans (Paneer), and Centratherum antihelminticum (Kali jeri) plant extracts against C. pseudotuberculosis. The study investigates whether these herbal extracts can serve as effective alternative treatments for CLA. The total of 120 samples were analyzed from infected sheep and goats using the disc diffusion method to determine the inhibition zones. The efficacy of two antibiotics ceftriaxone and tetracycline against herbal extracts was evaluated. The broth microdilution method uses minimum inhibitory concentration (MIC) for its measurements. The research requires further testing to confirm the veterinary applicability of plant extracts through extraction method optimization and dosage assessment and in vivo testing. The statistical method was used to analyze antimicrobial activity which showed Diameter of Zone of Inhibition results as mean±SE with statistical significance at probability level <0.05.

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INTRODUCTION

The economic system of Pakistan relies heavily on livestock which generates 14% of gross domestic product (GDP) while providing work opportunities to almost 8 million citizens who mainly reside in rural areas (Economic Survey of Pakistan, 2023). The livestock sector relies on sheep and goats because these animals deliver important outputs which include meat and milk and wool (Mazinani M., & Rude, B., 2020). The textile industry uses wool because these animals provide high-quality protein and essential nutrients through their meat and milk products (Habeeb, 2025). Pakistan ranks among the top milk-producing countries globally because small ruminant animals contribute significantly to the country's overall milk output (Nadeem M., & Ahmad, M., 2024). The economic advantages of sheep and goat farming arise from their fast-breeding cycles and their ability to produce low cholesterol meat and vitamin-rich meat and easily digestible milk that has small fat globules (Sindhura, A et al., 2023). Infectious diseases create major obstacles to sheep and goat production which results in economic losses for the industry (Aldridge et al., 2018). The primary infectious disease which affects sheep and goat production is caseous lymphadenitis (CLA) because it has major economic effects on the industry (Windsor, P. 2011). CLA is a chronic contagious disease which results from C. pseudotuberculosis and it causes major economic damages which decrease production of meat milk and wool while it also leads to poor animal quality and reduced breeding success and lower animal market prices (Dopuđ et al., 2025). The disease produces abscesses which develop in both superficial and internal lymph nodes and this condition leads to carcass condemnation, and it remains active in herds for extended periods.

C. pseudotuberculosis is a Gram-positive bacterium which exhibits multiple shapes and exists as a facultative intracellular pathogen that has a size range of 1.0 to 3.1 micrometers in length and 0.5 to 0.7 micrometers in width (Yaacob et al., 2021). The organism does not possess any capsule or flagella structures, yet it has fimbriae and shows its best growth at 37 degrees Celsius and pH 7.0 in anaerobic environments. The bacterium shows good growth on brain heart infusion agar and blood agar while its growth rate increases when yeast extract is added to the medium (Odhah, M. et al., 2017). The organism received its first identification from Edward Nocard in 1888 but scientists later placed it in the Corynebacterium genus because it shared morphological and cell wall characteristics with *C. diphtheriae* (Prygiel et al., 2022).

C. pseudotuberculosis exists in two biovars which scientists identify through their capability to reduce nitrate because biovar Ovis lacks this ability and primarily infects sheep and goats which results in CLA while biovar Equi possesses this ability and causes ulcerative lymphangitis and pigeon fever in horses (Karthik et al., 2026). The pathogenicity of this organism is mainly attributed to two virulence factors: phospholipase D (PLD) and a cytotoxic lipid cell wall (Domínguez et al 2024). PLD increases vascular permeability and enables bacterial spread and supports bacterial survival inside host body systems whereas the lipid-rich cell wall structure helps bacteria withstand host immune attacks and results in abscess development (Brito et al., 2019).

The transmission of *C. pseudotuberculosis* takes place through skin abrasions and through contaminated feed and soil and through fomites. The organism responsible for CLA maintains its presence in the environment for extended periods after pus discharge from ruptured abscesses which makes it a soil-borne disease. Insects and flies serve as mechanical vectors which help diseases spread between animals (Sarwar and Engineering, 2015). The disease causes affected animals to develop swelling that starts from their head and neck and limb areas and progresses until the swelling breaks open and releases thick yellowish-white pus. The development of internal abscesses results in chronic cough and respiratory distress and weight loss and diminished productivity (Mustaffa et al., 2025).

The traditional methods for diagnosing *C. pseudotuberculosis* infection depend on bacterial culture together with microscopy and biochemical testing but these methods require a long testing period which sometimes yields unclear results. The polymerase chain reaction molecular technique provides fast and precise identification results which laboratories now choose as their primary method for confirmation testing (Nassar et al., 2015). The treatment of CLA becomes difficult because the thick-walled abscesses block antibiotic delivery which makes antimicrobial treatment mostly ineffective 9 (McHenry, M. 2019). The standard medical approach applies surgical drainage together with antiseptic treatment whereas antibiotics function mainly to control secondary infections (Terré et al., 2011).

The search for new treatment methods has become more urgent because excessive and inappropriate antibiotic usage has led to the development of antimicrobial resistance. Approximately 78% of people worldwide use traditional plant-based medicines to treat infectious diseases according to global statistics (Wang et al., 2025). The medicinal plants Withania coagulans and Fagonia cretica show antibacterial properties because they contain bioactive phytochemicals which include alkaloids and flavonoids and tannins and saponins and terpenoids (Malavika et al., 2021). The plant-mediated process for creating silver nanoparticles functions as a sustainable method which shows improved ability to kill multidrug-resistant bacteria (Wahab, S., et al 2021). The economic impact of CLA together with the poor performance of standard antibiotics and rising antimicrobial resistance creates an urgent requirement to investigate plant-derived and other antimicrobial solutions for treating *C. pseudotuberculosis* (Mateo and Jiménez, 2022). The current research study aims to assess the antibacterial effectiveness of chosen medicinal plant extracts which provide a secure and affordable and environmentally friendly solution for controlling caseous lymphadenitis in small ruminants.

MATERIAL AND METHODS

The research study took place in the rural regions of Bahawalpur district which is in Punjab province of Pakistan. The area of Bahawalpur exists at geographical coordinates 29.41° N latitude and 71.67° E longitude and its hot weather conditions create optimal conditions for breeding small ruminants. The district has a population of approximately 3.8 million according to the 2023 census. The research team employed a cross-sectional study design to conduct their investigation. Researchers collected 120 pus samples from 60 sheep and 60 goats which showed clinical signs of caseous lymphadenitis in Bahawalpur district through their study as shown in figure 1. The researchers collected pus samples through aseptic methods from superficial lymph node abscesses while they documented animal and farm information through a structured questionnaire during the sampling process.

All the collected samples were transported to the Microbiology Laboratory, Department of Microbiology, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, for microbiological examination. The samples were kept at 4 °C until further microbiological examination. Isolation of the bacteria was done using conventional microbiological practices. The samples were inoculated onto nutrient agar medium and subsequently on blood agar at 37 °C (Patra et al., 2020).

Presumptive identification relied on the morphological characteristics in colonies, growth patterns, and hemolytic activity. Gram staining was used for microscopic examination to identify Gram-positive pleomorphic coccobacilli. Biochemical identification was achieved via catalase, urease, and nitrate reduction tests by standard procedures cited in Bergey's Manual of Determinative Bacteriology (AL-Joda et al., 2021).

According to the available literature, the seeds of *Withania coagulans, Fagonia cretica*, and *Centratherum antihelminticum* were collected from the authenticated herbal sources (Tanwar et al., 2024), as depicted in Figure 3. Furthermore, the plant materials were cleaned and then shade-dried, which were finally reduced to powder and subjected to maceration using ethanol, acetone, and water

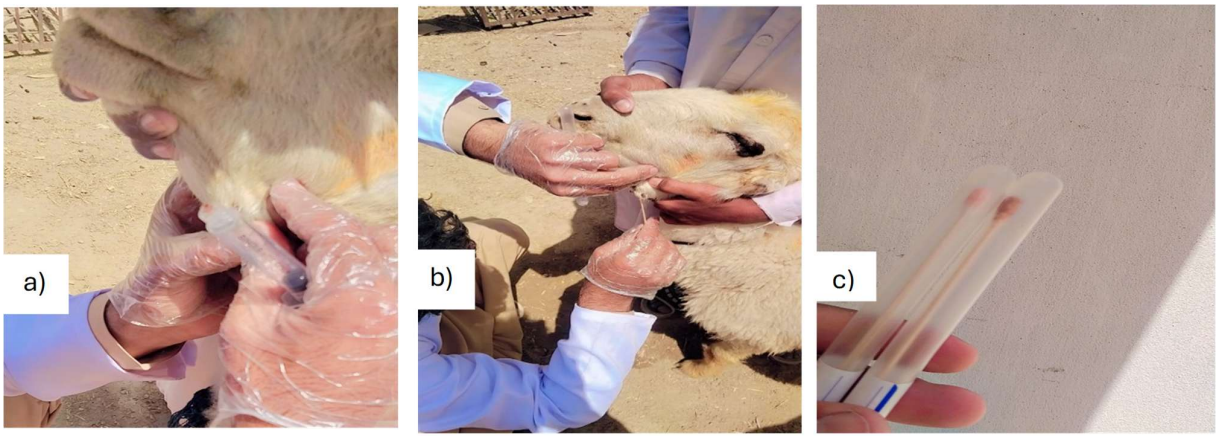

Fig. 1: lesions on sheep neck to take pus samples**, b)** Taking pus samples of lesions caused by *C. pseudotuberculosis* with the help of swabs, **c)** swab samples taken.

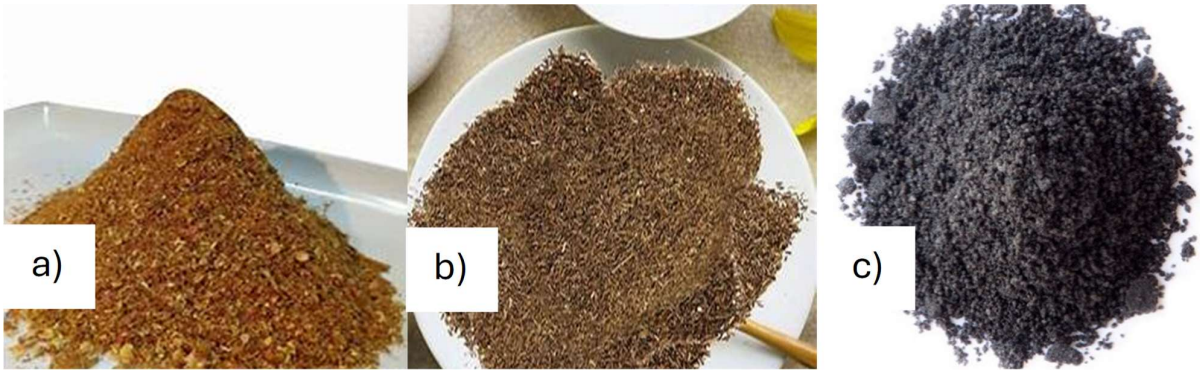

Fig. 2: a) Powdered *Withania coagulans* (Common name: Paneer), **b)** *Fagonia cretica* (common name: Sewar Booti) and **c)** *Centratherum antihelminticum* (Common name: Kaali Zeeri).

as solvents. Subsequently, the materials were filtered and stored at 4° C (Čertner, M., et al 2022). In addition, the standardized bacteria suspension of *C. pseudotuberculosis* was prepared from an overnight culture and then adjusted to the 0.5 McFarland standard, (≈1.5 × 10^8 CFU/mL) for antimicrobial testing (Mohamed et al., 2017).

The plant extracts were tested for antibacterial properties through the Kirby-Bauer disc diffusion method by using Mueller-Hinton agar (Hudzicki, J. 2009). The measured inhibition zones after incubating the samples at 37 °C for 24 hours and used control groups for their measurements. The minimum inhibitory concentration of plant extracts was determined against *C. pseudotuberculosis* through a broth microdilution method which they conducted in 96-well microtiter plates according to CLSI guidelines (Gemechu et al., 2013). The lowest concentration was recorded that stopped visible bacterial growth as the MIC.

RESULTS

The research study confirmed that *C. pseudotuberculosis* exists in sheep and goats from peri-urban and rural areas of Bahawalpur district. The study found that caseous lymphadenitis occurs widely among small ruminants in this region. The microscopic analysis showed Gram positive pleomorphic coccobacilli which matched the *C. pseudotuberculosis* body structure (Domenis et al., 2017). (Figure 3).

Researchers conducted an antibacterial activity assessment by testing 600 samples which included 300 samples from sheep and 300 samples from goats. The highest mean zone of inhibition which all treatments produced for sheep and goats occurred with distilled water which produced 11.87±3.09 mm and 11.63±3.08 mm results, and acetone delivered the next highest performance. Ceftriaxone showed its lowest antibacterial effectiveness against both species during all conducted experiments. The overall mean zone of inhibition across all samples was 10.16±3.17 mm which demonstrated that the tested treatments had moderate antibacterial effectiveness. Sheep showed a mean zone of inhibition of 10.02±0.18 mm, while goats exhibited a slightly higher mean of 10.31±0.18 mm. The confidence intervals for sheep and goats showed 95% overlap (Table 1) because their intervals included 9.67–10.36 mm and 9.97–10.66 mm ranges which proved the two species had no statistically significant difference.

Table 1: Comparison of Antibacterial Activity Across Solvent and Antibiotic Treatments

Solvent+ Antibiotics	Mean+ Std Error	95% Confidence Interval	
		Lower Bound	Upper Bound
Ethanol	9.750±.277	9.206	10.294
Acetone	10.692±.277	10.148	11.236
Distilled water	11.750±.277	11.206	12.294
Ceftriaxone	9.083±.277	8.539	9.627
Tetracycline	9.550±.277	9.006	10.094

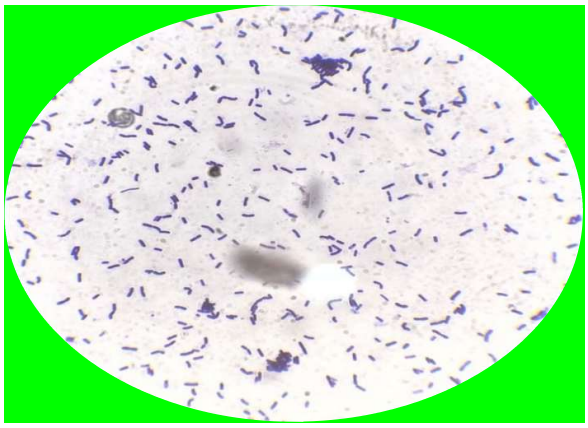

Fig. 3: Microscopic examination of *C. pseudotuberculosis*

Levene's test demonstrated that the species did not fulfill the requirement for equal variance because the test results showed an F value of 4.937 with a p value less than 0.001 Results demonstrated that animal species did not produce any substantial effects on their antibacterial activity as F value shows of 1.434 and p value of 0.232. The results demonstrated that treatment type had a strong impact on the zone of inhibition because F value reached 14.699 and p value fell below 0.001. The results did not show a significant interaction between the species of animal and the type of treatment, where (F: 0.963, and p: 0.428). The results obtained were significant, (F: 7.120, p<0.001), although the results obtained explained a low percentage of variance, where R^2 was 0.098.

The distilled water treatment showed the highest mean zone of inhibition which measured 11.75±0.28 mm while acetone and ethanol produced lower results of 10.69±0.28 mm and 9.75±0.28 mm respectively. The two antibiotics displayed reduced antibacterial effectiveness because ceftriaxone showed the lowest antibacterial effect which measured 9.08±0.28 mm (Figure 4).

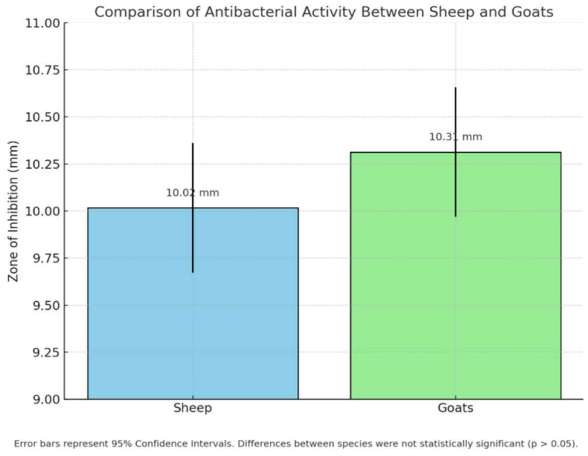

Fig. 4: Mean zone of inhibition (mm) of against bacterial isolates from sheep and goats.

The LSD tests conducted after the experiments demonstrated which treatment methods produced statistically significant results. The research found that distilled water showed antibacterial properties that were different from the effects of ethanol and acetone and ceftriaxone and tetracycline ($p < 0.01$). Acetone showed significant differences when compared to the two antibiotics. The two antibiotics ceftriaxone showed identical antibacterial effects against their target bacteria. Animal species did not affect the zone of inhibition (F=1.434, p=0.232), which proved that antimicrobial efficacy worked independently of species.

According to the results of this post-hoc analysis, distilled water was the best treatment for *C. pseudotuberculosis*, as both ceftriaxone and tetracycline were unresponsive to it. Acetone also had a greater effectiveness than either of the two antibiotics as well as ethanol having a level of effectiveness approximately equal to that of tetracycline. Both ceftriaxone and tetracycline exhibited no statistical difference between them thus indicating that solvent-based methods may be a viable substitute for conventional antibiotics.

Table 2: Post-Hoc Analysis of Antibacterial Efficacy Across Solvent and Antibiotic Groups

(I) Solvent + Antibiotic	(J) Solvent + Antibiotic	Mean Difference (I-J) +Std Error	Sig.	95%Confidence Interval for Difference
Ethanol	Acetone	-0.942±0.392	0.017	-1.711 to -0.172
Ethanol	Distilled water	-2.000±0.392	0.000	-2.769 to -1.231
Ethanol	Ceftriaxone	0.667±0.392	0.089	-0.103 to 1.436
Ethanol	Tetracycline	0.200±0.392	0.610	-0.569 to 0.969
Acetone	Distilled water	-1.058±0.392	0.007	-1.828 to -0.289
Acetone	Ceftriaxone	1.608±0.392	0.000	0.839 to 2.378
Acetone	Tetracycline	1.142±0.392	0.004	0.372 to 1.911
Distilled water	Ceftriaxone	2.667± 0.392	0.000	1.897 to 3.436
Distilled water	Tetracycline	2.200±0.392	0.000	1.431 to 2.969
Ceftriaxone	Tetracycline	-0.467±0.392	0.234	-1.236 to 0.303

DISCUSSION

The present research findings demonstrate their significance for studying *C. pseudotuberculosis* occurrence in sheep and goats throughout Bahawalpur district Pakistan. The area shows high occurrence of caseous lymphadenitis because C. pseudotuberculosis affects 35 percent of goats and 43 percent of sheep within the population. The economic impact of CLA in small ruminants has been documented in research from Brazil, Australia, and Mexico which confirmed its status as a major economic threat to their livestock industry (Williams et al., 2019). The high transmission rate of the pathogen requires development of cost-effective control solutions which need implementation on farms that lack sufficient control resources.

The current paper tested the antimicrobial effectiveness of three medicinal plants including Withania coagulans, Fagonia cretica and Centratherum antihelminticum against C. pseudotuberculosis. Traditional medicine uses these plants to treat infectious diseases therefore they were selected for study. The research team employed disc diffusion and minimum inhibitory concentration (MIC) tests to evaluate antibacterial activity, because this method serves as standard procedure for testing antimicrobial power. The observed inhibitory effects demonstrate that these plants contain bioactive compounds which inhibit C. pseudotuberculosis growth.

The research demonstrated that *Withania coagulans* possesses antibacterial properties which support existing knowledge about its antimicrobial capabilities. The traditional medical system has employed the plant for treating digestive problems and respiratory conditions and infectious diseases. Its anti-microbial effect on Gram-positive and Gram-negative bacteria including Staphylococcus aureus and Escherichia coli also justifies its therapeutic uses. The natural occurrence of *W. coagulans* together with its low toxicity function as a natural antibiotic replacement which farmers can use during agricultural production when veterinary drugs become unavailable. The extraction processes together with the formulation methods need standardization because they currently impede the implementation of large-scale operation. *Fagonia cretica* demonstrated strong antibacterial properties which proved effective against *C. pseudotuberculosis*. The plant demonstrates exceptional ability to adapt to arid and semi-arid environments because its people have traditionally used it to treat bacterial and parasitic and inflammatory health conditions. The current study shows that the plant exhibits strong antimicrobial properties, which support its recognition as a natural therapeutic agent. The anti-inflammatory and antioxidant effects of *F. cretica* can also serve as an additional factor to the effectiveness of this plant in nutrient chronic infections, including CLA, which is widely linked with chronic inflammation.

Centratherum antihelminticum demonstrated moderate antibacterial activity against *C. pseudotuberculosis*. The plant contains multiple bioactive compounds including flavonoids and alkaloids and saponins and tannins which display both antimicrobial and anti-inflammatory effects (Dar et al., 2023). The evidence shows that *C. antihelminticum* can be used as a supportive treatment for CLA even though the plant showed only minimal antibacterial activity. The use of plant-based antimicrobials requires alternative treatment methods because *C. pseudotuberculosis* develops thick-walled abscesses that block antibiotic penetration.

Plant-based antimicrobials provide hospitals with a method to decrease their need for conventional antibiotics while these plants serve as a solution to the growing problem of antimicrobial resistance (AlSheikh, H. et al., 2020). Herbal extracts function as combination therapies with antibiotics which enhance treatment effectiveness while reducing bacteria strength and shortening recovery time (Coates et al., 2020). Researchers need to investigate the pharmacokinetics and bioavailability and toxicity and effectiveness of these plants before veterinarians can start using them in animal healthcare.

The research findings demonstrate that *Withania coagulans* and *Fagonia cretica* and *Centratherum antihelminticum* show potential to function as natural alternatives to traditional antibiotics for treating *C. pseudotuberculosis* and use in CLA treatment for small ruminants. The existing research needs to be expanded through additional studies which will establish secure and effective sustainable plant-based treatments that can protect livestock health.

Conclusion

The experiment provides a solution which demonstrates effective methods to treat caseous lymphadenitis which is caused by Corynebacterium pseudotuberculosis in sheep and goats. The results demonstrate that plant solvent extracts especially those created through distilled water and acetone extraction methods show strong antibacterial properties against C. pseudotuberculosis which makes them effective substitutes for standard antibiotics. The study results show that ceftriaxone and tetracycline which doctors commonly prescribe failed to work effectively because of the growing issue of antibacterial resistance which requires scientists to create new therapeutic solutions. The research results show that there is no significant difference between the antibacterial response of sheep and goats which means that treatment success depends on the chosen antimicrobial agent instead of the animal species and thus the treatment can be applied to all animal types. Traditional construction methods which produce medicinal plants show that they can provide practical and affordable yet sustainable options for modern veterinary practice. The in vitro results show positive results, but scientists need to conduct more research about active phytochemical substances and optimal extraction methods and about how phytochemicals function and their safe dosage on living organisms. The plant extracts and antibiotics should be studied together to identify their combined effects which would enhance treatment results while reducing resistance development. The research demonstrates that plant-based antimicrobials effectively control CLA and other bacterial infections in livestock which leads to improved animal health and reduced need for antibiotics and sustainable livestock farming practices.

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Ethics Statement: All the research procedures were conducted according to guidelines regarding use of animal for research purpose.

Author's Contribution: AH, RA: Idea and design; MM, AZ: Data collection and writing draft; ZS and MA: reviewing and editing of the manuscript.

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