



(RESEARCH ARTICLE)



Anti-microbial activity Test against *E. coli* using Cow's Urine

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Abstract

The accelerating global concern over antibiotic resistance necessitates a focused search for effective, non-conventional antimicrobial agents. This study investigated the antibacterial potential of cow urine a substance historically revered and widely utilized in traditional medicine specifically targeting the clinically relevant opportunistic pathogen, *Escherichia coli* (*E. coli*). Utilizing the established agar well diffusion method, different concentrations of fresh cow urine were evaluated for their capacity to inhibit *E.coli* growth. The experimental results were significant, clearly demonstrating a prominent Zone of inhibition surrounding the wells treated with cow urine. This evidence confirms that active compounds within the urine effectively suppress the propagation of the bacterial culture. Size of the inhibition zone suggests the presence of potent antimicrobial components. These findings validate traditional usage and establish cow urine as a promising, accessible source for developing novel therapeutic agents. Future research is essential to isolate *Escherichiae* and characterize the active constituents and fully elucidate their specific mechanism of action against *E. coli* cell structures.

Keywords: *Escherichia coli*; Anti-Microbial Activity; Cow's Urine; Bacterial Culture

1. Introduction

It represents mother earth, as it is a source of goodness and its milk nourishes all creatures. Krishna, a central Hindu deity, is often portrayed in stories recounting his life as a cowherd and referring to him as the child who protect cows. *Escherichia coli* is a type of bacteria that lives in many places in the environment, including the gastrointestinal system of humans and warm-blooded animals, where it is part of the gut microbiota. Some strains of *E. coli* can be administered as pro-biotics and are known to have a positive effect on host health. However, some strains can be pathogenic, causing intestinal and extraintestinal infections in humans as well as animals. *E. coli* is hence a bacterium with a wide range of different natural types of strains, each with its own set of features. Because of its unique qualities, such as simplicity of handling, availability of the entire genome sequence, and capacity to grow in both aerobic and anaerobic conditions, *E. coli* is also a popular bacterium for laboratory research and biotechnology. So, *E. coli* is considered to be the utmost widely utilized microbe in the field of recombinant DNA technology, and it is used in a wide range of industrial and medical applications. And the main thing is also that people used to consume Cow's urine as a sign of holiness after taking bath in the early morning and that may be the main reason why they used to healthy as compared to our present generation, as the significance of Cow's urine is much higher but people need to understand and people should be aware of their own roots so they might dig up some even more interesting and unknown benefits of it.

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1.1. Aetiology

- *E. coli* is part of commensal intestinal flora and is also found on the floors of hospitals and long-term care facilities.
- *E. coli* is the most common gram-negative bacteria in the human gastrointestinal tract.

However, when found outside of the intestinal tract, *E. coli* can cause urinary tract infections (UTI), Pneumonia, Bacteremia and Peritonitis, among others.

1.2. Epidemiology

Escherichia coli results in intestinal illness as well as infection outside of the intestine. Intestinal illness caused by *E. coli* is caused by one of five subtypes, and they are identified according to their O and H antigens.

2. Review of literature

The utilization of products derived from the cow, particularly urine, is deeply embedded in certain traditional medical systems, holding spiritual and therapeutic significance that precedes modern pharmaceutical development. This context is essential for understanding the continued interest in cow urine as a subject of scientific inquiry.

2.1. Role of *Bos indicus* Products in Ayurveda (Panchgavya Chikitsa)

In traditional Indian culture, the cow (*Bos indicus*) is regarded as a sign of spirituality, and its products form the basis of a holistic treatment modality known as Panchgavya Chikitsa, or Cowpathy. This traditional system relies on five fundamental components derived from the cow: urine (Gomutra), milk, ghee (clarified butter), curd, and dung. Cow urine is considered the paramount component among these.

The therapeutic application of Gomutra has an extensive history within Indian traditional medicine, specifically ayurveda. Ancient Ayurvedic texts, including the Sushruta Samhita and Ashtanga Sangraha, describe Gomutra as an effective medicinal substance, often characterizing it as the best of all animal urines, possessing "innumerable therapeutic properties". Its high status is reflected in the appellations "Sanjivani" (life restorer) and "Amrita" (nectar or elixir of life) conferred upon it in Ayurvedic literature.

2.2. Global Folk Medicine and Traditional Administration

The practice of consuming urine, known as urophagia, is not exclusive to Indian traditions; it was used in several ancient cultures for healing and cosmetic purposes and is still practiced in some parts of the world today. Cow urine specifically finds medicinal use in regions outside of India, including Myanmar and Nigeria. In Nigeria, for instance, a traditional remedy for convulsions in children includes cow urine mixed in a concoction with garlic, tobacco, rock salt, and lemon basil juice.

Historical methods of administration in India were varied, extending beyond consumption to include practices such as streaming the urine directly onto a sick person's face.

2.3. Trace Element Analysis via ICP-MS

Chemical analysis has identified a wide array of inorganic components. Cow urine contains 24 types of salts, and important minerals such as iron, calcium, phosphorus, potassium, nitrogen, ammonia, manganese, and sulfur have been quantified.

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyses have confirmed the presence of various macro- and micro-elements, including sodium (Na), calcium (Ca), iron (Fe), magnesium (Mg), potassium (K), zinc (Zn), chromium (Cr), and trace amounts of Gold (Au). The detection of trace gold, referred to in Ayurvedic texts as Swarn kshar (aurum hydroxide), provides a measurable element corresponding to a traditionally cited substance believed to improve immunity. This modern analytical confirmation of traditional concepts represents a crucial step in validating the material composition referenced in ancient texts.

2.4. Review of International Patents for Cow Urine Distillate (CUD)

The industrial and scientific validation of CUD's bio enhancing property is underscored by the issuance of two United States Patents (US Patent Nos. 6,896,907 and 6,410,059). This is a significant milestone, shifting CUD from a traditional remedy to a formally recognized pharmacological agent.

These patents specifically protect the use of CUD or its isolated bioactive fractions as "activity enhancers and availability facilitators" for a broad range of molecules, including anti-infective agents, anti-cancer compounds, and essential nutrients. The patented applications extend across human, animal, and agricultural health.

2.5. Antioxidant Capacity and Hepatoprotection

Cow urine exhibits significant antioxidant properties, which are crucial for neutralizing oxidative stress and scavenging free radicals within the body. This effect is chemically supported by the presence of uric acid and various vitamins (A, C, E), along with bioactive compounds such as phenolics and flavonoids.

Preclinical animal studies have confirmed this protective action. In models of paracetamol-induced liver damage in rats, cow urine protected against hepatotoxicity by restoring normal levels of liver enzymes and enhancing the activity of intrinsic antioxidant enzymes (Achliya et al., 2004). This hepatoprotective property suggests a strong potential for CUD as a supplement for managing chronic conditions.

3. Challenges and Limitations

Despite extensive historical use and promising preclinical research, the integration of cow urine (Gomutra) or its distillate (CUD) into mainstream medical practice faces significant challenges and limitations, primarily rooted in the requirements for modern pharmacological standardization, safety, and evidence-based efficacy.

3.1. Deficit in Human Clinical Validation

The most substantial barrier to the widespread medical acceptance of Gomutra is the critical lack of rigorous, large-scale human clinical trials.

3.1.1. Unsubstantiated Claims

While traditional texts and preclinical studies attribute broad therapeutic potential (e.g., anti-cancer, anti-infective) these claims largely lack confirmation from scientific backing as required by conventional regulatory standards.

3.1.2. Low Clinical Research Volume

A review focusing on Gomutra literature noted a significant disparity, identifying only seven clinical studies compared to 15 animal studies and numerous in vitro analyses.[6] This scarcity of clinical evidence means that many of the observed benefits remain confined to the laboratory or animal model.

3.1.3. Need for Confirmation

Medical experts and researchers underscore that comprehensive, well-designed human trials are an essential step before cow urine can be recommended for the general population or for the treatment of serious health conditions.

3.2. Safety, Toxicity, and Contamination Concerns

While traditional medicine often regards cow urine as non-toxic [8], modern analytical and toxicological studies have raised specific safety concerns regarding its preparation and commercial use.

3.2.1. Risk of Pathogenic Contamination

Raw cow urine, the form often used in traditional practice, poses fundamental risk of containing pathogenic bacteria, leading experts to caution against its unapproved consumption.[5]

3.2.2. Toxicity of Combined Formulations

Safety is highly dependent on formulation. For instance, a cow urine extract combined with *Nicotiana tabacum* leaf was found to induce hepatotoxicity (liver damage) and renal impairment in animal models, demonstrating that extracts are not universally safe.

3.2.3. Recent Trends

Advanced Molecular Characterization and Diagnostics: Metabolomics and Proteomics

Scientific efforts are heavily focused on employing high-resolution analytical technologies to map the molecular inventory of cow urine. This approach is fundamental to identifying the active pharmacological agents and distinguishing the chemical signature of cow urine from that of other biofluids.

3.2.4. Comprehensive Metabolomic Profiling and Bioactive Molecule Identification

Recent studies have utilized advanced analytical platforms, notably Gas Chromatography–Mass Spectrometry (GC–MS) and Proton Nuclear Magnetic Resonance (Proton -NMR) spectroscopy, to conduct comprehensive, comparative metabolomic profiling across various cow breeds. This detailed delineation of urinary metabolites represents a critical opportunity for bio-based innovation and new product development, enabling the linkage of specific molecules to potential therapeutic functions.

4. Materials required

- Volumetric flask
- Pond water Sample
- Sterilized Petri-plates
- Mac Conkey's agar
- Inoculation Loop
- Beaker
- Agar-agar (Solidifying agent)
- Test-tubes
- Incubator
- Refrigerator
- Cow's Urine sample
- E. coli sample
- Micro-pipette
- Autoclave
- Distilled water
- Gloves

5. Methodology

5.1. Sample Preparation

Took the Cow's Urine sample in a transparent container for testing about 24 hours ago.

5.2. Bacterial Culture

Pure culture of Escherichia coli was obtained and maintained on Mac Conkey's agar The bacterial suspension was prepared to achieve standardized inoculum concentration for consistent results.

5.3. Antimicrobial Testing

The well diffusion method was employed to evaluate antimicrobial activity. Three sterile petri-plates containing MacConkey's agar medium were prepared and inoculated with *E. coli* culture using spread plate technique.

Two petri-plates were chosen and wells were made using Cork borer and sample is filled in one well and another well is left empty and considered as Control.

5.4. Incubation and Measurement

Both plates were incubated at 37°C for 24 hours. Following incubation, zones of inhibition around wells and discs were measured using standard measuring scales. The diameter of clear zones was recorded in milli- meters.

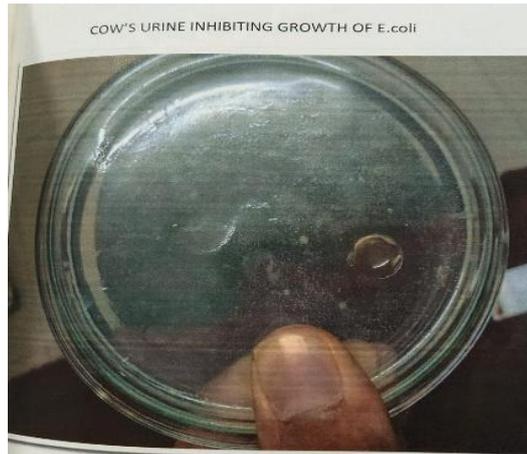


Figure 1 Cow's Urine Inhibiting Growth of *E.coli*



Figure 2 Pipetting of Sample

6. Results

The antimicrobial activity was assessed based on the formation and measurement of zones of inhibition around the wells.

6.1. Zone Of Inhibition Results

Zone of Inhibition was Observed measuring about 15mm and which confirmed the Sensitiveness of *E. coli* against Cow's Urine Sample.

7. Discussion

The present study investigates the antimicrobial potential of fresh cow urine against *Escherichia coli*; a common pathogenic bacterium associated with several gastrointestinal infections. Fresh cow urine was collected under aseptic conditions and directly assessed for its antibacterial efficacy using the agar well diffusion method. The obtained results revealed a distinct zone of inhibition around the wells treated with cow urine, indicating its significant inhibitory effect on the growth of *E. coli*. This suggests that certain bioactive compounds naturally present in cow urine, such as volatile fatty acids, phenolic derivatives, and urea, may contribute to its antimicrobial properties.

8. Conclusion

The present investigation clearly demonstrates that fresh cow urine possesses significant antimicrobial activity against *Escherichia coli*, one of the most prevalent pathogenic bacteria responsible for various intestinal and urinary tract infections. The formation of a distinct zone of inhibition confirms that cow urine contains potent bioactive compounds capable of suppressing bacterial growth. These findings scientifically validate the traditional claims regarding the medicinal value of cow urine in ancient Ayurvedic literature, which described it as a natural disinfectant and immune booster.

The antimicrobial efficacy observed in this study may be attributed to the synergistic action of several organic and inorganic constituents present in cow urine, such as volatile fatty acids, phenolic compounds, minerals, and urea.

These compounds are believed to interfere with bacterial cell membrane integrity, enzyme function, and metabolic pathways, ultimately leading to growth inhibition or cell death. The results indicate that fresh cow urine could serve as a cost-effective and eco-friendly alternative to certain synthetic antimicrobial agents, particularly in rural and resource-limited settings where antibiotic resistance poses a major challenge.

Nevertheless, the study also highlights the importance of further research to isolate, purify, and characterize specific bioactive molecules responsible for the antibacterial activity. Advanced analytical techniques such as GC-MS, HPLC, and NMR spectroscopy could be employed in future studies to identify these active components. In conclusion, this work contributes to the growing evidence that natural biological resources, especially cow urine, can be effectively utilized for developing sustainable, low-cost, and safe antimicrobial formulations, paving the way toward greener alternatives in modern medicine and biotechnology.

Scope for further enhancement

The present study opens several avenues for future research to enhance the understanding and practical applications of cow urine as a natural antimicrobial agent. Further work can be directed toward the isolation and characterization of specific bioactive compounds responsible for antibacterial activity using advanced analytical tools such as GC-MS, FTIR, and NMR spectroscopy. This will help identify the key molecules that contribute to microbial inhibition and support the development of purified formulations with consistent potency.

Additionally, studies involving different breeds of cows, varying diets, and stages of lactation can be conducted to assess the influence of biological and environmental factors on the antimicrobial efficacy of cow urine. Testing its activity against a broader spectrum of pathogenic bacteria and fungi will also help establish its potential as a broad-range biotherapeutic.

Future enhancement could include developing nano formulations or combining cow urine extracts with plant-based compounds to improve stability, bioavailability, and targeted delivery. Toxicity studies and in vivo experiments are also necessary to confirm its safety and therapeutic viability. Overall, systematic research and standardization efforts can transform cow urine from a traditional remedy into a scientifically validated and commercially valuable natural antimicrobial resource.

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