Antibacterial and Antifungal Activity of Cow Urine

Dr. Anjali Soni
Associate Professor
Department of Biotechnology,
Veer Narmad South Gujarat University, Surat
***Email ID: anjalisoni@vnsgu.ac.in**

Ms. Mehal Patel
Research Scholar
Department of Biotechnology,
Veer Narmad South Gujarat University, Surat
Email ID: mehalpatel01@gmail.com

Dr. Preeti Sharma
Associate Professor
Department of Biotechnology,
Veer Narmad South Gujarat University, Surat
Email ID: preetisharma@vnsgu.ac.in

Ms. Upexa Patel
Research Scholar
Department of Biotechnology,
Veer Narmad South Gujarat University, Surat
Email ID: upexapatel1313@gmail.com

Abstract:

The increase in the occurrence of multidrug-resistant infections emphasizes the need for exploring alternative antimicrobials. In this study, different forms of cow urine which includes fresh cow urine, sterile cow urine and photo-activated cow urine are tested for their antibacterial, antifungal activity along with the extract from leaves of Saraca asoca (Asopalav). Agar well diffusion method was used for assessment of the microbial activity. Cow urine in its fresh state exhibited a substantial antibacterial activity. Sterile cow urine and photo-activated cow urine had a minor inhibition but the action was significantly increased when mixed with S. asoca extract against various pathogens. As confirmed by a total phenolic analysis, the greatest amounts of phenols associated with antibacterial action were detected in fresh cow urine. Results have validated the ancient use of cow urine as a natural antibacterial agent.

1. Introduction:

The cow is considered as the most important animal in the Vedas, ancient Hindu literature (Meena et al., 2019). Five significant materials obtained from cows are referred to as panchgavya: urine, ghee, curd, milk and dung. All five compounds are used medicinally. The importance of Panchgavya is mentioned in Ayurveda. It was used to cure a wide range of human illnesses (Rana & De, 2013).

"Antimicrobial resistance is a global public health concern in the modern period(Iwamoto et al., 2019). According to estimates, bacterial AMR led to million deaths globally in 2019 and directly caused 1.27 million"(Sagar et al., 2024). Drug-resistant diseases are mostly caused by improper and excessive use of antibiotics in humans, animals, and plants. All nations, regardless of location or economic status, are affected by AMR. Its origins and negative consequences are exacerbated by poverty and inequality, especially in low- and middle-income countries (Murray et al., 2022). AMR is not limited to bacteria; drug-resistant fungus, such as Candida auris and other species, pose a particularly dangerous hazard to patients due to their multidrug resistance, even when paired with antifungals (Dolnicar et al., 2015).

In the short term, high AMR impacts healthcare by threatening to render modern medicine impossible because more complex surgeries and chemotherapy would be limited and imposed enormous economic burdens. (Sakalauskienė et al., 2025). All these challenges underscore the need for alternative antimicrobial approaches. Cow urine has a unique significance in Ayurveda. It has been called the nector from God(Sachdev et al., 2012). Cow urine therapy can be followed for the following conditions: indigestion, thyroid, stomach ache, asthma, psoriasis, constipation, jaundice, gynecological issues, anemia, hemorrhoids and vitiligo. Cow urine acts as an antimicrobial against different types of bacteria (Meena et al., 2019).

1.1 Composition of CU

Cow urine is actually an excretion liquid, made up mainly of just harmless waste products filtered from the cow's body (Randhawa & Sharma, 2015). The major component is water and remaining portion is urea, enzymes, minerals, hormones and salts (Pandit et al., 2024). Molecules known for antimicrobial and germicidal activity include salts of calcium, creatinine, aurum hydroxide, urea, carbolic acid, phenols and manganese (Pant et al., 2024)(Ashara & Shah, 2016). The cow is thus often described as a kind of `living-pharmacy from where we can directly get all the medicinal substances (Meena et al., 2019).

1.2 MECHANISM OF ACTION OF CU

Urine produced by the cow has both volatile and non-volatile components that account for its antimicrobial properties. Several compounds like creatinine, urea, swarnkshar (gold hydroxide), phenols, carbolic acid, calcium and manganese help to exhibit its germicidal effect. The presence of urine peptides and amino acids increases the hydrophobicity of the surface of bacterial cell, hence enhancing the bactericidal activity. Additionally, it is thought that cow urine increases macrophage phagocytic capacity, boosting immunity (Pullaiah, 2023)(Meena et al., 2019).

Fresh cow urine has higher contents of phenols as compared to the distillation product (cow urine distillate, CUD), therefore the fresh cow urine produces greater antibacterial activity against microbial growth. When they are photo-activated generate many volatile organic and inorganic compounds. Photo activated cow urine (PhCU) is more acidic compared to fresh cow urine, which is also a factor that likely enhances its bactericidal potential. In addition, an inorganic component, such as phosphorus, chloride and dimethylamine were also believed to be involved in this antimicrobial mechanism. Altogether, these properties assist cattle urine to block the cultivation of pathogenic microorganisms(Meena et al., 2019).

1.3 Origin and Source of Cow Urine

Gomutra (Cow urine) has been considered as a bio resource with medicinal, agricultural and cultural significance in Ayurveda, ethnic medicine and ethnoveterinary practices. But cow urine is not a uniform product in terms of composition and potential, with variation based on factors including breed, age, physiological condition, diet and the management system. Gir (Gyr) cattle are important indigenous Indian breeds due to their high milk yield and good adaptability towards tropical stress along with recognized medically useful urine and other by-products.

The Gir or Gyr is an important breed of Zebu native to India. The Gir is well-liked in Brazil and several other South American countries as it is also environment-friendly cattle breed of Bos indicus qualities with resistance to high temperatures and tropical diseases. The breed is most noted for its ability to produce milk with average lactation yields of around 2,110 liters and exceptional cases over 5,000 liters in organized farms. They have also been involved in the breeding of North American Brahman and Brazilian Girolando, as Gir is one of the breeds with high milk yield among indigenous bovines. Synonyms: Bhadawari, Bhodali, Desan, Gujarati, Kathiawari, Sorthi and Surati breed. The breeding tract is known by the name of the Gir forest, geographical home land of the breed and which consists of Amreli, Bhavnagar, Junagadh and Rajkot districts of Gujarat. In Ayurveda and ethnoveterinary medicine, gomutra (cow urine) has been recognized for its many medicinal benefits, in addition to milk. Urine from pregnant cows is thought to contain unique nutrients and hormones. According to Ayurveda many diseases can all be treated with gomutra (cow urine).

1.4 Fresh, Sterilized, and Photo activated Cow Urine

Cow urine possesses antibacterial, antioxidant and immunomodulatory properties; however, the effectiveness may differ depending on processing methods. In the present study we investigated three types of cow urine samples; fresh cow urine, sterilised urines and photo activated cow urine. The entire spectrum of the volatile and non-volatile compounds with antimicrobial and therapeutic potential, are retained in fresh cow urine. Sterilized cow urine (autoclaved or filtered) devoid of microbial contamination is prepared for experimental usage. This crystallizing event is photodecomposition of Urea and its applications in soil fertility. The results show that photochemical reactions occur in the presence of urea, and uric acid from cow composite leads to changes in ROS and Peroxides on UV or sunlight exposure. These changes enhance both antibacterial and antifungal, and even anticancer activities. Consequently, freshly collected cow urine receives natural potency with the sterilization increasing safety but potentially leading to loss of efficacy and photo activation significantly improves therapeutic use due to reactive oxygen species mediated mechanisms. This comparative analysis makes it clear that the bioactivity of cow urine is directly related to processing, and reproducible standardized processes need to be utilized for biomedical uses.

1.5 Synergistic Antimicrobial Activity

The therapeutic impact of cow urine can be enhanced by combining it with medicinal herbs that have a potent antibacterial effect. The sacred evergreen Ashoka (Saraca asoca) tree, which is a member of the Fabaceae family, has important medical, cultural, and spiritual significance (Borokar & Pansare, 2017). S. asoca has diverse pharmacological applications, which include a wide range of clinical and medicinal benefits. It possesses antioxidant, anti-inflammatory properties as well antibacterial CNS depressant effects Further investigation of the isolate and crude extracts from S. asoca has indicated that its constituents also have antidiabetic and anticancer activities besides cardio-vascular or gynecological efficacies.

This synergy is particularly beneficial against drug-resistant bacteria, when conventional antibiotics are ineffective. Our results demonstrate that a combination of cow urine (photo activated or fresh) with Saraca asoca extracts/ juice is likely to be an effective bio enhancer. These synergistic preparations, that is potent extracts at lower concentrations of botanicals, which improves medicinal effect and possibly even reduces side effects. Therefore, the co-administration of cow urine with medicinal plants like Saraca asoca would be an innovative approach to develop an herbal-based natural antibacterial medicine that reputably needs further pharmacological validation.

2. Materials and Methods

2.1 Collection of Cow Urine:

Cow urine from four distinct breeds was collected at the Goushala in Surat (Vaijnath Goushal). The study's experimental cow was a well-mannered seven-year-old Gir cow that was given a regular immunization vaccination schedule and fed a consistent diet that included equal amounts of concentrates and grass.

2.2 Preparation of Concentrates:

Fresh cow urine: It was collected in sterile plastic bottles and transported to the laboratory for determination. (Ghosh & Biswas, 2018); **Sterile cow urine:** The urine sample was autoclaved; **Photo -activated cow urine:** fresh cow urine was kept in sun light for 72 h under sealed glass bottle. All samples were Filtered through Whatmann No. 1 filter paper in order to eliminate precipitated material and debris, then stored at 4ºC in sterile plastic bottle prior use. Cow urine was screened to have no microbial contamination prior evaluation of antibacterial activity (Ghosh et al., 2018).

2.3 Preparations of Cow Urine Concentrate:

After giving these different treatments to the cow urine sample, cow urine is poured into differently labelled crucibles and put it in Hot Air Oven at 60-70ºC for 2 days. After this, they were weighed differently by weighing machine and make it soluble in 1 mL DMSO and use it further in antimicrobial assays(Shah et al., 2011).

2.4 Plant Material:

Collection of plant material: Medicinal plant Saraca asoca (Asopalav) used in the study was collected from the backyard of the home. Green leaves are used for the assay. It was subjected to different processes to prepare its extracts. Preparation of plant extract: The leaves were properly washed with water and then air dried in hot air oven at 60-80ºC for 30-40 minutes (Hata et al., 2024). By taking it out and crush it finely, which increases surface area of powdered particles of leaves and give better result during soxhlet method for plant extract(Wang & Weller, 2006). Method of extraction: Plant extraction is carried out using the Soxhlet technique. Run the soxhlet for three to four cycles, or until the extract no longer changes colour (Dauda et al., 2022).

2.5 Antimicrobial Activity Assay:

The test cultures (Proteus vulgaris, Bacillus subtilis Staphylococcus aureus, and Escherichia coli for the bacteria or fungi: Aspergillus fumigatus) were obtained from Department of Biotechnology of Veer Narmad South Gujarat University. Well Diffusion Method was used to assess the antimicrobial activity of cow urine(Docherty & Kulpa Jr, 2005).

2.6 Estimation of Total Phenolic Content:

This estimation is carried out by Folin-Ciocalteau method (Kumar et al., 2023).

3. Results

3.1 Comparative evaluation of the Antimicrobial efficacy of Fresh, Sterile and photo activated cow urine against Pathogenic bacteria.

Antimicrobial activity of Fresh, Sterile and photo activated cow urine was determined against Escherichia coli (E. coli), Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris with different concentration (0.16, 0.32, 0.64,1.32 mg/ml) using the agar well diffusion method where results reported as diameter of zone of inhibition(mm).

3.1.1 Fresh cow urine

E. coli showed a clear response based on concentration. Inhibition zones ranged from 2 mm at 0.16 mg/mL to 10 mm at 1.32 mg/mL. S. aureus was highly susceptible, with a maximum zone of 14 mm at 1.32 mg/mL. However, there was a modest reduction at 0.64 mg/mL, with an 8-mm zone. P. vulgaris was only suppressed at the highest dose, measuring 4 mm at 1.32 mg/mL. B. subtilis showed progressive suppression as concentrations rose, reaching 13 mm at 1.32 mg/mL [Figure 1].

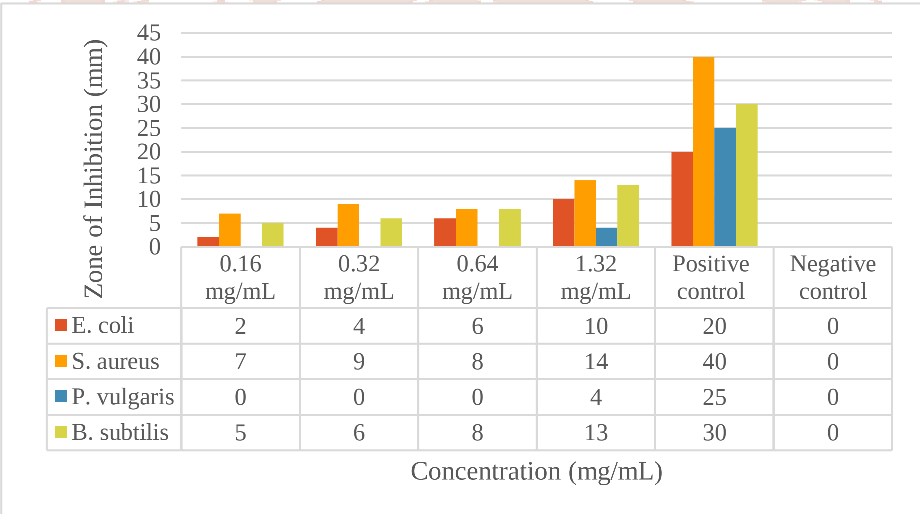
3.1.2 Sterile cow urine

Sterilization shows the significant decline in antimicrobial activity compared to fresh urine. inhibition of E. coli was minimal, ranging from 1 mm to 4 mm. S. aureus remained extremely sensitive, with inhibition zones of up to 11 mm. Inhibition of P. vulgaris ranging from 2 mm to 9 mm. Inhibition of B. subtilis, progressively rising from 6 to 9 mm [Figure 2].

3.1.3 Photo-activated cow urine

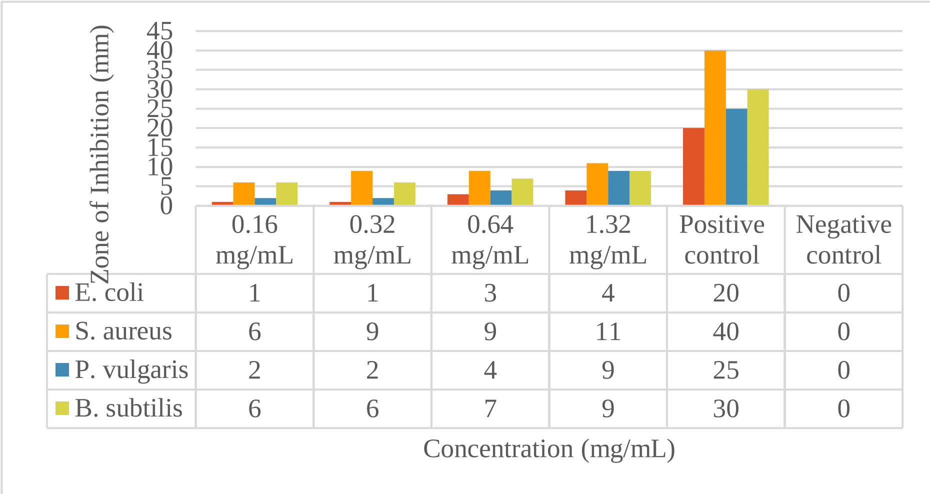
By photo activation, the antimicrobial efficacy of cow urine got enhanced notably against S. aureus and P. vulgaris. Inhibition of E. coli was only observed at higher concentrations. Inhibition zone ranging from 3 mm to 14 mm. Progressive inhibition of P. vulgaris was observed from 3 mm to 7 mm. At lower doses, B. subtilis had no inhibition, and only 8 mm at the maximum dose [Figure 3].

The comparative analysis of cow urine variations revealed unique antibacterial responses in test organisms. S. aureus was the most susceptible, with consistently wide zones of inhibition (7-16 mm) across all treatments, demonstrating that it is particularly sensitive to the bioactive elements of cow urine independent of the processing method. B. subtilis also demonstrated moderate to significant inhibition, with the greatest impact (13 mm) reported in fresh cow urine, indicating that heat- or light-sensitive chemicals may contribute to its action. E. coli showed lesser inhibition overall, with a maximum of 10 mm in fresh samples, although photo-activated and sterile preparations had lowered effects, indicating organism-specific resistance. surprisingly, P. vulgaris responded minimally to fresh and sterile variations but showed increased sensitivity to photo-activated cow urine (up to 7 mm), indicating that photo-activation may boost the efficiency of antimicrobial chemicals against this organism. Overall, the results obtained indicate that even though fresh cow urine has wide antimicrobial action, photo activation may selectively boost effects against some resistant pathogens, while sterilization decreased overall activity [Figure 4].

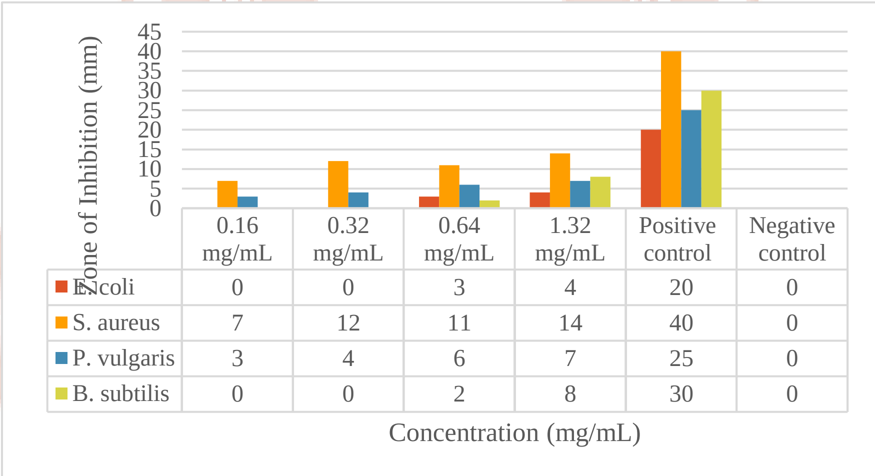


	0.16 mg/mL	0.32 mg/mL	0.64 mg/mL	1.32 mg/mL	Positive control	Negative control
E. coli	2	4	6	10	20	0
S. aureus	7	9	8	14	40	0
P. vulgaris	0	0	0	4	25	0
B. subtilis	5	6	8	13	30	0

[Figure 1: Effect of the fresh cow urine on the growth of different bacterial strain.]

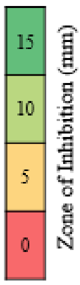


[Figure 2: Effect of the sterile cow urine on the growth of different bacterial strain.]



[Figure 3: Effect of the photo-activated cow urine on the growth of different bacterial strain.]

Organism	Fresh CU-0.16	Fresh CU-0.32	Fresh CU-0.64	Fresh CU-1.32	Photo. CU-0.16	Photo. CU-0.32	Photo. CU-0.64	Photo. CU-1.32	Sterile CU-0.16	Sterile CU-0.32	Sterile CU-0.64	Sterile CU-1.32
B. subtilis	5	6	8	13	0	2	8	6	6	7	9	9
E. coli	4	6	4	10	0	0	3	4	1	1	3	4
P. vulgaris	0	0	0	0	4	3	6	7	2	2	4	9
S. aureus	7	9	8	14	7	12	11	14	6	9	9	11



[Figure 4: Heat map: Antimicrobial activity of cow urine variants]

3.2 Assessment of Antimicrobial potential of Saraca asoca (Asopalav) plant extract against Pathogenic bacteria

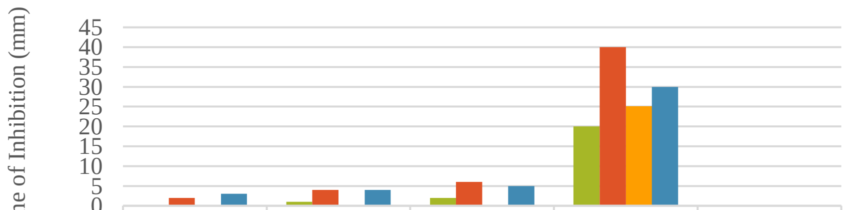
Antimicrobial effects of Asopalav (Saraca asoca) plant extract were studied on four bacteria: E. coli, S. aureus, P. vulgaris, and B. subtilis using three different concentrations: 0.003 mg/ml, 0.006 mg/ml, and 0.009 mg/ml. Results indicate dose-dependent inhibition zones of growth for S. aureus and B. subtilis, suggesting antibacterial action. On the other hand, E. coli shows less sensitivity, with growth inhibition at higher concentrations of 1mm and 2mm, while there is no growth inhibition for P. vulgaris. The positive control used here is Streptomycin Sulphate, showing growth inhibition ranging from 20–40mm, while water acts as the negative control and shows no growth inhibition. Based on the above results, it is possible to conclude that the antibacterial effect shown by the extract of Asopalav is selective and more potent against S. aureus and B. subtilis than Gram-negative bacteria [Figure 5].

3.3 Assessment of antifungal potential of cow urine and plant extracts against Aspergillus fumigates

Antifungal activity of the various cow urine, Saraca asoca extract, and their combination was determined by using the agar well diffusion technique against Aspergillus fumigatus. The results have been expressed in terms of zones of inhibition (mm).

3.3.1 Antifungal activity of different samples of cow urine against Aspergillus fumigates

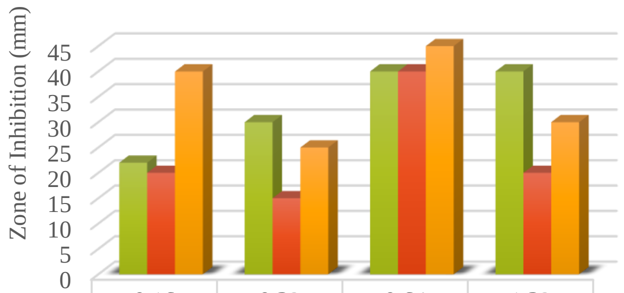
Among the various cow urine formulations tested, the photo-induced cow urine formulation displayed the highest activity against fungi at the lowest concentration of 0.16 mg/mL, providing a 40 mm zone of inhibition. The activity of fresh cow urine against fungi was found to be concentration-dependent and provided the maximum zone of inhibition of 40 mm at 0.64 mg/mL and 1.32 mg/mL concentrations. The inhibition zones for sterile cow urine were moderately high, with a maximum zone of inhibition of 40 mm being achieved at 0.64 mg/ml [Figure 6].



	0.003 mg/mL	0.006 mg/mL	0.009 mg/mL	Positive control	Negative control
E. coli	0	1	2	20	0
S. aureus	2	4	6	40	0
P. vulgaris	0	0	0	25	0
B. subtilis	3	4	5	30	0

Concentration (mg/mL)

[Figure 5: Antibacterial activity of the plant extract against selected bacterial strains.]



	0.16 (mg/mL)	0.32 (mg/mL)	0.64 (mg/mL)	1.32 (mg/mL)
Fresh Cow Urine	22	30	40	40
Sterile Cow Urine	20	15	40	20
Photo-Activated Cow Urine	40	25	45	30

[Figure 6: Antifungal activity of different variants of cow urine]

3.3.2 Antifungal activity of different samples of Saraca asoca (Asopalav) plant extract against Aspergillus fumigates

The antifungal activity against A. fumigatus exhibited by the aqueous extract of Saraca asoca was relatively less when compared to others. Zones of 3 mm and 5 mm inhibition were obtained at concentrations of 0.003 mg/mL and 0.006 mg/mL, respectively. These findings suggest that the extract has antifungal activity; nevertheless, it had lower potency than the cow urine extracts.

The activities of various combinations of cow urine with S. asoca extract were tested against fungi after incubation for 12 h and 24 h. The ratio of FU/A after 12 h proved to have more stable antifungal activity, with inhibition zones of 12 mm present at all ratios (90/10, 80/20 and 60/40) tested. The inhibition zones of sterilized cow urine–plant extract combination (SU/A) and the photo-activated cow urine–plant extract combination (PA-U/A) were 10 to 12 mm and 9 to 12 mm, respectively.

Nevertheless, after incubating for 24 h, a significant loss in antifungal activity was found for all combinations. FU/A combination exhibited the highest level of activity, with inhibition zones 3–4 mm. SU/A showed weak activity with inhibition zones decreasing to 1–7 mm, and PA-U/A even less active with the values of inhibition zones between 1–3 mm. The 60/40 ratio of SU/A combination did not show any inhibition after 24 h [Figure 7].

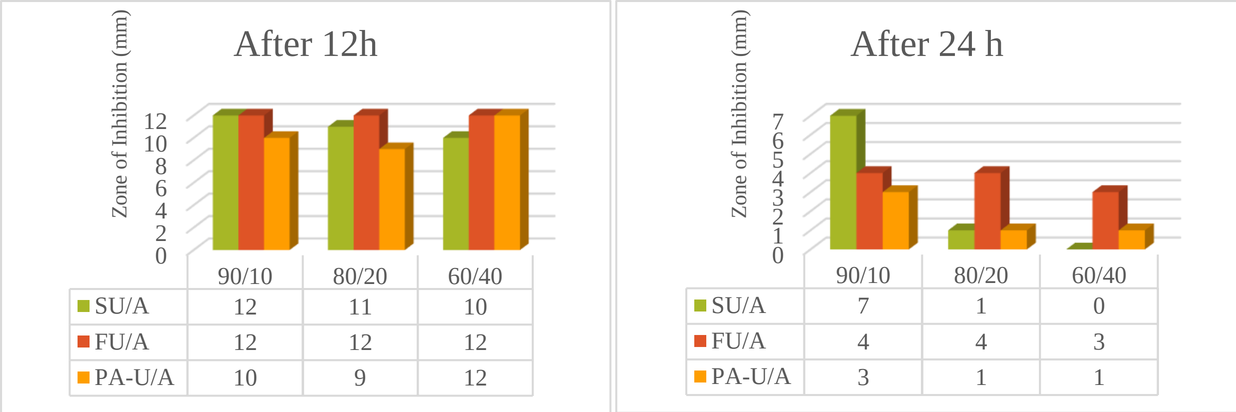


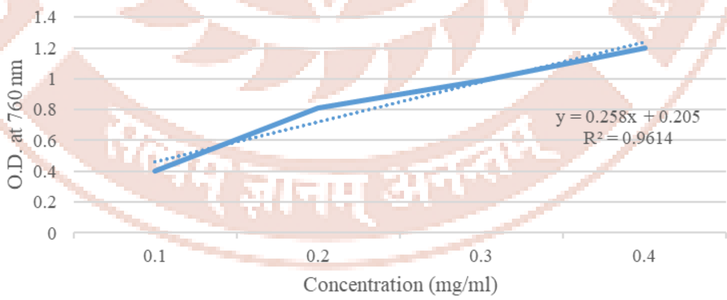
Figure 7: Comparative antifungal activity of sterile cow urine (SU), fresh cow urine (FU), and photo-activated cow urine (PA-U) in combination with *Saraca asoca* extract against *Aspergillus fumigatus* after 12h and 24h

These results indicate that although each cow urine sample possessed a high antifungal activity against A. fumigatus individually, the blended (cow urine + S. asoca extract) samples became less effective antifungals, especially after long-term co-culture with fungal cells. The gradual decline in activity over a period of time might be due to loss of stability or even antagonistic relationship between the bioactive compounds from cow urine and the extracts from plant.

3.4 Estimation of total phenolic content in different types of Cow urine sample

3.4.1 standard curve

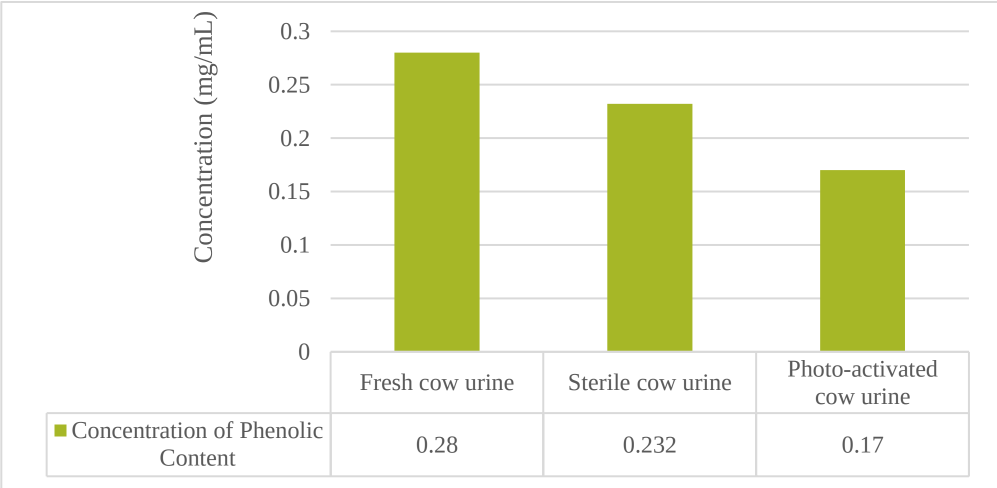
To determine the total phenolic content of cow urine samples, a standard curve was created using various gallic acid concentrations. The Folin-Ciocalteu technique was used to measure



[Figure 8: Gallic Acid standard curve]

the absorbance values at 760 nm. The absorbance rose in direct proportion to the gallic acid concentration, as seen in Figure 8, demonstrating a positive linear connection between concentration and optical density. The test samples' total phenolic content was determined using the standard curve, and the findings were represented as gallic acid equivalents (GAE).

Fresh cow urine has the greatest computed phenolic content followed by sterile cow urine and lowest found in photo-activated cow urine. These results suggest that photo-activation and sterilizing procedures may lower the amount of phenolic chemicals in cow pee. The breakdown or transformation of phenolic compounds during sterilization and photo-activation may be responsible for the decrease in phenolic content seen following these procedures [Figure 9].



[Figure 9: Total phenolic content of different variants of cow urine]

4. Discussion:

This study strongly proves the antimicrobial efficacy of different preparations of cow urine namely fresh, sterile and photo activated against various pathogens causing diseases in humans, along with the synergistic effects obtained by combining the preparations with extracts of Saraca asoca. Fresh cow urine has significantly high antimicrobial compared to other two preparations; this is evident from inhibition zones which were found to be bigger, especially in case of Staphylococcus aureus and Bacillus subtilis.

The sterilization process of cow urine, although vital for removing microbes, led to a marked reduction in the level of antimicrobial activities. This was caused by the deterioration of some volatile components because of autoclaving, thus leading to low phenol content. However, the photo activated cow urine exhibited selective increases in antimicrobial activity against some of the microorganisms tested, such as P. vulgaris.

Antimicrobial activity of Saraca asoca leaf extracts is more potent in killing Gram-positive bacteria. Similarly, S. asoca extracts combined with cow urine (especially fresh or photo activated) exhibited higher activity against Aspergillus fumigatus suggesting a synergistic action. These findings suggest that a combination strategy using conventional cow urine mixtures along with extract of plant may be effective at treating microbial infections, especially as the resistance of bacteria to antibiotics is on the rise.

Mechanistically, cow urine acts as an antimicrobial agent because of the properties of some bioactive constituents such as uric acid, creatinine, urea, hydroxide and mainly phenolic compounds which act by destroying microbial cell wall and preventing enzyme activity and immunity. The different levels of biological action manifest themselves due to the various chemical and phenolic contents of the cow urine samples, which is a vital biochemical foundation

Conclusively, the observed results can be considered to scientifically prove that cow urine has activity (fresh more) as well as substantiating many traditional perceptions for cow urine having antimicrobial activity. Cow urine synergises with Saraca asoca that which can be used for antimicrobial therapy, especially around the issue of resistant microorganisms.

References:

- Ashara, K. C., & Shah, K. V. (2016). Cow's urine: an incredible aqueous phase. *Global Journal of Biotechnology & Biochemistry*, *11*(2), 145–152.
- Behera, B., Singh, A., Kumar, S., & Mishra, S. (n.d.). *Ethnobotanical, phytochemical and pharmacological potentials of Saraca asoca (Roxb.) WJ de Wilde*.
- Borokar, A. A., & Pansare, T. A. (2017). Plant profile, phytochemistry and pharmacology of Ashoka (Saraca asoca (Roxb.), De. Wilde)-A comprehensive review. *Int. J. Ayurvedic Herb. Med*, *7*(2), 2524–2541.
- Dauda, J. A., Jacob, A. D., Ogaji, O. D., Haruna, S., Idowu, O. L., Are, C. T., Abdullahi, A. S., Ocheme, G. W., Olupinyo, O., & Oteno, F. (2022). Synergetic Assessment of the Elemental Profile, Phytoconstituents, Antioxidant and Antibacterial Efficacy of the Methanolic Extracts of Persea americana and Daucus carota Fruits. *FUDMA JOURNAL OF SCIENCES*, *6*(6), 97–104.
- Docherty, K. M., & Kulpa Jr, C. F. (2005). Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chemistry*, *7*(4), 185–189.
- Ghosh, T., & Biswas, M. K. (2018). Evaluation of antibacterial and antifungal activity of cow urine against some seed borne microflora. *International Journal of Current Microbiology and Applied Sciences*, *7*(5), 1714–1727.
- Ghosh, T., Biswas, M. K., & Guin, C. (2018). Efficacy of cow urine in wheat (Triticum aestivum) production as plant growth promoter and antifungal agent. *Journal of Pharmacognosy and Phytochemistry*, *7*(3), 485–493.

- Hata, F. T., da Silva, D. C., Yassunaka Hata, N. N., Pavinatto, M. da S., de Queiroz Cancian, M. A., Macedo, R. B., Ventura, M. U., Resende, J. T. V. de, & Spinosa, W. A. (2024). Using bokashi and cow urine as organic low-cost amendments can enhance arugula (Eruca sativa L.) agronomic traits but not always total polyphenols and antioxidant activity. *Horticulturae*, *10*(2), 155.
- Iwamoto, K., Pedersen, H. B., Tello, J. E., Lo Fo Wong, D., & Robertson, J. (2019). Cross-programmatic consultation on the role of primary care in the responsible use of medicines and the reduction of antimicrobial resistance. *Expert Review of Anti-Infective Therapy*, *17*(2), 75–78.
- Kumar, R., Kaushik, J. K., Mohanty, A. K., & Kumar, S. (2023). Identification of bioactive components behind the antimicrobial activity of cow urine by peptide and metabolite profiling. *Animal Bioscience*, *36*(7), 1130.
- Meena, M., Saini, S., Gurjar, T. G. R., & Meena, O. P. (2019). Go mutra (Cow urine) and its uses: An overview. *Journal of Entomology and Zoology Studies*, *7*(3), 1218–1222.
- Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., Han, C., Bisignano, C., Rao, P., & Wool, E. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655.
- Pandit, N., Deoghare, C., & Chaudhury, S. K. (2024). A review on the green chemistry perspective of multipurpose use of cow urine. *Pure and Applied Chemistry*, *96*(10), 1499–1522.
- Pant, L., Thapa, S., Dahal, B., Khadka, R., & Biradar, M. S. (2024). In silico and in vitro studies of antibacterial activity of cow urine distillate (CUD). *Evidence-Based Complementary and Alternative Medicine*, *2024*(1), 1904763.
- Pullaiah, T. (2023). *Phytochemical Composition and Pharmacy of Medicinal Plants: 2-Volume Set*.
- Rana, R., & De, S. (2013). In vitro antimicrobial screening of cow urine-a potential natural antimicrobial agent. *International Journal of Bioassays*, *2*(2), 436–439.
- Randhawa, G. K., & Sharma, R. (2015). Chemotherapeutic potential of cow urine: A review. *Journal of Intercultural Ethnopharmacology*, *4*(2), 180.
- Sachdev, D. O., Gosavi, D. D., & Salwe, K. J. (2012). Evaluation of antidiabetic, antioxidant effect and safety profile of gomutra ark in Wistar albino rats. *Ancient Science of Life*, *31*(3), 84–89.
- Sagar, S., Singh, A., Bala, J., Chauhan, R., Kumar, R., Bhatia, R. K., & Walia, A. (2024).

Insights into cow dung-based bioformulations for sustainable plant health and disease management in organic and natural farming system: a review. *Journal of Soil Science and Plant Nutrition*, *24*(1), 30–53.

- Sakalauskienė, G. V., Malcienė, L., Stankevičius, E., & Radzevičienė, A. (2025). Unseen enemy: mechanisms of multidrug antimicrobial resistance in Gram-negative ESKAPE pathogens. *Antibiotics*, *14*(1), 63.
- Shah, C. P., Patel, D. M., Dhami, P. D., Kakadia, J., Bhavsar, D., Vachhani, U. D., Trivedi, M. N., & Joshi, V. J. (2011). In vitro screening of antibacterial activity of cow urine against pathogenic human bacterial strains. *Int J Curr Pharm Res*, *3*(2), 91–92.
- Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*(6), 300–312.