COW URINE DISTILLATE AS A SUSTAINABLE MICROBIAL CULTURE MEDIUM

A Research Project

Submitted by

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UNDER THE GUIDANCE OF

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VIVEKANAND COLLEGE, KOLHAPUR

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"Dissemination of education for Knowledge, Science and culture"

- Shikshanmaharshi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

VIVEKANAND COLLEGE, KOLHAPUR

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DEPARTMENT OF MICROBIOLOGY (PG)

CERTIFICATE

OF

RESEARCH PROJECT COMPLETION

This is to certify that Ms. **SHARAYU PRADEEP BHOSALE** studying in M. Sc. part II, Sem- IV at Vivekanand College, Kolhapur (An Empowered Autonomous Institute) has sincerely completed research project work entitled **"COW URINE DISTILLATE AS A SUSTAINABLE MICROBIAL CULTURE MEDIUM"** during academic year 2025-26.

Dr. Savita D. Mali

Research Project Guide

24/4/26

24.04.26

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1.0 Introduction

Cow urine (Gomutra) is a natural biological fluid excreted by healthy cows and has been traditionally valued in India for its medicinal, agricultural, and environmental benefits. In recent years, it has gained scientific attention due to its rich composition and diverse microbiological potential. Cow urine contains water, urea, minerals, salts, enzymes, volatile compounds, and other bioactive constituents that provide a favorable environment for the growth and activity of various microorganisms.

The chemical composition of cow urine primarily consists of approximately 95% water. The remaining 5% contains about 2.5% urea and 2.5% a mixture of minerals, salts, hormones, and enzymes. Some of the important minerals and compounds found in cow urine include calcium, chloride, creatinine, magnesium, potassium, sodium, sulphate, uric acid, ammonia nitrogen, allantoin, and various vitamins, such as A, B, C, D, and E. It also contains bioactive compounds such as phenols, carbolic acid, and volatile organic compounds. Cow urine has a pH of 7.4-8.4 and exhibits antimicrobial properties, primarily due to urea and uric acid.

Cow urine (CU), commonly referred to as 'Gomutra' in traditional Indian literature, has been an integral component of Ayurvedic medicine for centuries. It is a complex biological fluid composed of water, urea, creatinine, enzymes, minerals, volatile fatty acids, phenolic compounds, and other bioactive molecules (Pant et al., 2024).

Its composition varies with the cow's diet, age, health, and breed. Modern scientific studies have increasingly recognized cow urine as a bioactive agent with diverse applications in microbiology, agriculture, and nanotechnology (Agrawal, et al.,2023).

Presently, we face a global public health crisis, as infectious diseases top the list. It is widely accepted among clinicians, medical researchers, microbiologists and pharmacologist that antibiotic resistance contributes significantly to this problem; data on consumption and resistance to morbidity and mortality is quantitative nuclear (S.Raad 2013). Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness, higher expenditures and a great risk of death. There is a dire need for the development of new antimicrobial agents with sensitivity impacts. The rational designing of novel drugs from traditional medicines to treat these difficult-to-treat infections offers a new prospect for the modern healthcare system.

Nair (1999) stated that a combination of an antibiotic and cow's urine distillate enhanced the antimicrobial effect of the antibiotic. CIMAP [Central Institute of Medicinal and Aromatic Plants] Lucknow, GVAK [Go-Vigyan Anusandhan Kendra] and CSIR [Council for Scientific and Industrial Research] laboratory have researched on cow's urine distillate and an antibiotic showed that distillate helped to improve the efficiency of the antibiotic and acts as a bioenhancer or drug facilitator.

Hemdari (1987) suggested that the Gomutra is well known for its therapeutic action on urinary tract infection and diabetes. It is also widely used for its aphrodisiac [Vajikaarna Rasayana] properties. In India, scientific studies have shown that 'Cow's urine distillate" has properties that enhance the antibiotic and antimicrobial effects of medicines and got a US patent for it.

The cow [Bos indicus or Bos indicus] has placed at high pedestal for enormous usages of their valued harvests like the dairy products [colostrum, milk, clarified butter and cheese] and animal waste like dung and urine (Gupta, et al.,2016.) such products are the major ingredients in 'Panchgavya' that has been widely used tonic, immune booster, and other therapeutic formulation in Ayurveda for millennia (Joshi, etal,2015]) they are also have huge sources of many bioactive substances, responsible for their diverse pharmacological actions (Joseph et al.,2020)

An anti-cancer drug extracted from cow urine and developed by an affiliate of the RSS has got a third US patent for its anti-genotoxicity properties. The same extract developed by RSS-backed Go-Vigyan Anusandhan Kendra had earlier got the US patent as a bio–enhancer with antibiotics and anti-cancer drugs. Research for the drug whose brand name is 'Kamdhenu Ark' was carried out jointly by Kendra and the

National Environment Engineering Research Institute [NEERI]. A composition by an Indian scientist, using cow urine distillate to enhance the antimicrobial effect of the antibiotic present in the formulation, has been granted a US patent.

Antimicrobial activity of CU from both indigenous and hybrid breeds against *E. coli, Salmonella typhi, Proteus vulgaris, S. aureus, Bacillus cereus, Staphylococcus epidermidis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas fragi, Streptococcus agalactiae, Enterobacter aerogenes, Aeromonas hydrophila, Micrococcus luteus, Streptococcus pyogenes, Streptomyces aureofaciens, Lactobacillus acidophilus* and *Bacillus subtilis*, and *Leishmania donovani* has been observed in various studies. In these studies, the antimicrobial activity of CU was found to be

comparable with ofloxacin, ciprofloxacin, ampicillin, chloramphenicol, nalidixic acid, rifampicin, tetracycline, streptomycin, cefpodoxime and gentamycin in different studies (Gurpreet R., et al., 2015)

Cow urine distillate (CUD) is often preferred over raw cow urine in research because it offers better purity, stability, and consistency. Distillation removes impurities, microbial contaminants, and suspended solids, yielding a clear and sterile liquid enriched with volatile bioactive compounds. Unlike raw urine, which varies in composition and decomposes quickly, CUD is more standardized and has a longer shelf life, making it suitable for antimicrobial, antiviral, and plant growth studies. Its clarity and stability also ensure more reliable and reproducible experimental results. (Pant, et al., 2024)

In recent years, growing scientific interest has highlighted the potential of cow urine as a valuable natural resource with diverse biological and agricultural applications. Its unique chemical composition, coupled with its eco-friendly and cost-effective nature, makes it an attractive alternative to synthetic chemicals and conventional agents. Further systematic research and experimental validation can help establish its role in sustainable agriculture, biomedicine, and environmental management.

Savita Jandaik (2015) conducted a study to determine the antifungal activity of three different concentrations (5, 10, and 15%) of cow urine against three fungal pathogens (*Fusarium oxysporum, Rhizoctonia solani, and Sclerotium rolfsii*) isolated from infected plants of Methi and Bhindi that showed symptoms of damping off and wilting disease by poison food technique. The extent of growth of test fungi in plates poisoned with cow urine was less when compared with the control plates. Among these

concentrations, cow urine at 15% concentration was most effective. When the three fungal organisms were compared, maximum growth suppression was observed in *Fusarium oxysporum* (78.57%) at 15% concentration of cow urine, followed by *Rhizoctonia solani* (78.37%) and *Sclerotium rolfsii* (73.84%). They concluded that the cow urine has antifungal activities, and the inhibitory activity can be used in the control of fungi. The nutritional effect of cow urine on plant growth was also tested with *Trigonella foenum-graecum* (Methi) and *Abelmoschus esculentus* (Bhindi) plants and the chlorophyll and protein content was also estimated.

The study also investigates the use of cow urine as a plant growth enhancer and antimicrobial agent, drawing upon its historical significance in Hindu culture as depicted in ancient texts. Cow urine was tested on five plant types: maize, wheat, lemon grass, tukmaria, and methi, using concentrations of 0%, 5%, 15%, and 25%. The key findings revealed that a 5% cow urine concentration promoted maximum plant growth, while 25% exhibited significant antibacterial and antifungal properties against *Escherichia coli* and *Aspergillus* fungi. Gas Chromatography Mass Spectroscopy (GCMS) identified 16 compounds in cow urine; of these, several showed antibacterial and antifungal effects while others acted as plant growth enhancers. The study concludes that cow urine can effectively enhance plant growth and possesses antimicrobial properties, supporting its continued use in traditional medicine. (Karthikeya et al.,2019)

During the past few years cow urine therapy has provided promising and authentic results for the treatment of cancer, a deadly malady which is being faced by the mankind and the incidences of which are ever increasing in the current scenario of changed lifestyle and food habit's along with exposure to predisposing factors of carcinogens

such as tobacco chewing, smoking, alcohol intake, environmental pollutants, occupational health hazards etc. Anti-cancer potential of cow urine therapy has been reflected by several case reports, success stories and practical feedback of patients for the treatment of cancer. Cow urine enhances the immunocompetence and improves general health of an individual; prevents the free radicals and acts as an anti-ageing factor; reduces apoptosis in lymphocytes and helps them to survive; and efficiently repairs the damaged DNA, thus is effective for cancer therapy (K. DHAMA1, et.al.,2005).

Thus, various studies have been done and are continually being done on the uses of cow urine. In conclusion, cow urine is a valuable natural resource with immense potential in agriculture, medicine, and microbiology. Its unique chemical composition and antimicrobial properties make it useful for promoting plant growth, controlling pathogens, and supporting sustainable practices. Scientific studies continue to validate many of the traditional uses of cow urine, opening new avenues for developing ecofriendly biofertilizers, antimicrobial agents, and bioremediation strategies. With proper standardization and research, cow urine can contribute significantly to sustainable development and modern scientific innovations.

As per the above-mentioned benefits and uses, we decided to study cow urine for its microbial applications.

2.0 Review of Literature

2.1 Antimicrobial activity of cow urine:

Cow urine, traditionally known as *Gomutra* in Ayurveda, has been used in India for centuries as a natural therapeutic and disinfectant agent. In recent years, scientific studies have increasingly focused on exploring its antimicrobial potential against a wide range of pathogenic microorganisms. Cow urine is a complex biological fluid containing water (about 95%), urea, minerals, salts, hormones, enzymes, and volatile compounds such as phenols and indoles, which may contribute to its antimicrobial properties.

Study analyzes the study analyses the antibacterial and antifungal activity of Cow Urine Distillate against the clinical pathogenic microorganisms. Antibacterial activity of Cow

Urine Distillate (5, 10 and 15µl) was analyzed against the *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumoniae and Salmonella typhi*. Maximum antibacterial activity was observed in *Pseudomonas aeruginosa* (7.06±0.05, 8.08±0.18, and 10.4±1.23 mm in diameter, respectively) and *Salmonella typhi* (6.3±1.23, 8.06±0.17, and 10.4±1.2 mm in diameter, respectively). (Arunkumar Sathasivam, et.al. 2010)

Antimicrobial activity has also been demonstrated against some resistant strains, including multidrug-resistant (MDR*) Escherichia coli and Klebsiella pneumoniae*. Antimicrobial action is further enhanced by its immune-enhancing and bio enhancing effects on some antibiotic drugs. Antifungal activity was comparable to amphotericin B. CU also has anthelmintic and antineoplastic action. (G.al. 2015.)

Gram-negative bacteria are generally more resistant compared to the gram-positive ones. The resistance could be due to the permeability barrier provided by the cell wall. (Rinkal Rana, et.al., 2013).

A combination of photo-activated and its binary combinations was determined against seven bacterial strains. Photoactivated cow urine has shown MIC value 0.25 ml/ml against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Lactobacillus acidophilus* (ATCC 53103), and *Micrococcus luteus* (ATCC 9341), while it was found to be 0.125 ml/ml against *E. coli* (ATCC 25922). Inhibition zone diameters obtained in the presence of photoactivated cow urine and its binary combinations were found to be much larger than those of antibiotic drugs. (RK Upadhyay, et.al.,2012)

The antimicrobial activity of cow urine is a consequence of the feeding habit. The metabolites of plant origin with several bioactivities are eliminated through urine and are responsible for their antimicrobial nature. Secondly, the plethora of peptides generated from the activity of endogenous proteases on protein shed from different parts of tissues also finds its way to the urine. Some of these sequences possess antimicrobial activity due to their amino acid composition. (R Kumar, et.al.,2023)

Cow urine has antibacterial and antioxidant activities and varies in potency according to altitudinal and climatic differences. Hence, cow urine from the subalpine zone has better antibacterial and antioxidant activity than that of lower altitudinal climatic zones, as concluded by (R Joshi, et.al.,2019).

Cow urine extract, hexane, chloroform, ethyl acetate, alcohol, methanol, and aqueous fractions of Pongamia pinnata Linn seed were tested against *X. oryzae pv.* for their antibacterial activity. Streptomycin sulphate (30 µg) and dimethyl sulfoxide (DMSO, 15 µL) are used as positive and negative controls. All the extracts and fractions were effective and showed a 10 to 13 mm zone of inhibition. Phytochemical analysis also showed the presence of terpenoids, quinine, coumarin, tannin, and phenol, with flavonoid available in higher quantity (1.56 mg kg-1) (AM Murugan, et.al.,2015).

The present study was undertaken to determine antibacterial, antifungal, and anthelmintic activity of Cow urine concentrate (CUC), which is obtained by complete evaporation of cow urine. The antibacterial activity was tested against Gram-positive and Gram-negative bacteria by the disc diffusion method. Antifungal activity was tested against species of *Aspergillus* by the Agar well diffusion method. Anthelmintic activity was studied using the adult Indian earthworm model. Marked inhibition of Gram-positive bacteria were observed by the CUC. Inhibition of fungi was found to be dose-dependent. Among fungi tested, *A. niger* was more affected than others. In the anthelmintic assay, concentration-dependent mortality of worms was observed, and the effect of CUC was found to be superior compared to the standard drug Piperazine citrate. The antimicrobial and anthelmintic activity of CUC may be due to the presence of constituents in it. The CUC could be used in the treatment of diseases caused by pathogenic bacteria, opportunistic fungi, and parasitic helminths. Further studies on the isolation of inhibitory components and in vivo experiments are to be carried out. (P Kekuda T.R. et.al.,2010).

Studies on the Antibacterial effects of Cow urine distillate have revealed it to be toxic to and inhibitive to the growth *of Staphylococcus aureus, E. coli, Pseudomonas species, Bacillus subtilis, Proteus vulgaris, Streptococcus species, Klebsiella pneumonia, and Salmonella typhi* in culture. Antifungal activity on *Aspergillus* species was also observed. Various experiments show fungicidal effect against various species of *C. tropicalis, Aspergillus malassezia, and C.glabrata.* CU (Cow urine) inhibits the growth of *Malassezia* fungi (90-95%), which is responsible for causing dandruff for a longer time (4-5 days). Also, CU shows a significant effect on various microorganisms, which is responsible for different diseases in crops. Study found that Lemon Juice extract and Neem leaves extract are less effective than (CU) 21. CUC (cow urine concoction) 5% showed maximum antifungal activity against *A. niger* (93%), *A. oryzae* (92.67%) and *A. flavus* (83%) (Meena M, et.al.,2019).

2.2 Cow urine-mediated nanoparticles synthesis

In every facet of nanotechnology, the buzzing of nanotechnology has been flourishing at a remarkable rate in recent decades (Jain et al. 2011). The bio-nanoparticles can work efficiently as fertilisers, pesticides, and fungicides in the field of agriculture and horticulture. The biological products can reduce metal ions to metal nanoparticles. The biological products are eco-friendly, less toxic, cost-effective, and also have sacred molecules that enhance the quality and quantity of products in agriculture and horticulture (Govardhan et al. 2014). The biomaterials like plant extracts, animal secretions, and microorganisms can be used to synthesize the AgNPs (Kumar et al. 2009, Ahmad et al. 2003, Shahverdi et al. 2007, Jha et al. 2009, Atul et al. 2008, Lee et al. 2013).

Velmurugan et al., (2011). Cow urine has a pool of sources like nitrogen, phosphorus, potassium, calcium, magnesium, Sulphur, chlorite, iron, silicon, lactose, carbolic acid, urea, aromatic acids, arum hydroxide, hippuric acid, protein, and creatinine. The urea vitamins in urine are A, B, C, D, and E, and gold acids (Pathak & Kumar 2003). It also works as a plant hormone to enhance the growth of the plant and correct micronutrient deficiency in plants (Pradhan et al. 2018, Sahu et al. 2016).

Silver oxide (Ag2O) nanoparticles were produced using cow urine as a reducing agent through combustion at 500°C, producing ~20 nm spherical particles. The nanoparticles demonstrate excellent photocatalytic degradation of methylene blue (83.63% in 4 hours), strong antibacterial activity against pathogenic bacteria, and yellow photoluminescence suitable for LEDs. The gomutra-mediated method is faster, more economical, and environmentally friendly compared to conventional synthesis techniques, offering a scalable approach for water treatment and biomedical applications. (S.P. Vinay et al. 2019).

Synthesised silver nanoparticles using cow urine, creating 47.8 nm particles that showed strong antimicrobial activity against antibiotic-resistant bacteria (E. coli and MRSA) through cell wall destruction and DNA damage. The nanoparticles proved biocompatible with blood cells at safe concentrations, offering a cost-effective and eco-friendly alternative for treating resistant bacterial infection (A.S. Santosh et al. 2021).

A rapid one-pot synthesis of silver nanoparticles (AgNPs) using fermented cow urine combined with ultrasonication, achieving particle formation in just 1 minute. The synthesised nanoparticles (11-20 nm in size) were characterised using various analytical techniques and demonstrated significant antibacterial activity against multiple bacterial pathogens and antifungal activity against the fungus *Fusarium oxysporum,* suggesting potential applications in cosmetics and wound dressings. Meghnath Prabhu et al., (2014) synthesised silver nanoparticles (AgNPs) using honey and cow urine (gomutra) via a hydrothermal method and characterised them using UV-Vis's spectroscopy and scanning tunnelling microscopy. The AgNPs were tested for antibacterial activity against Pseudomonas species isolated from contact lenses, showing significant inhibition of bacterial growth and reduction of biofilm formation, with potential applications in preventing contact lens-related eye infections (N. Jain et al., 2019).

Synthesized silver nanoparticles (22-40 nm size) from goat, cow, and buffalo urine, conjugated them with the fungicide Mancozeb, and tested their antifungal activity against the fungus *Colletotrichum gloeosporioides* that causes anthracnose disease in fruits and vegetables. The Mancozeb-conjugated nanoparticles showed significantly enhanced antifungal activity (114-146% more effective than fungicide alone), with goat urine-derived particles being the most potent. This approach allows for reduced fungicide use while maintaining or improving disease control effectiveness (S.N. Raghvendra et al. 2020).

The green synthesis of various nanoparticles (including silver, zinc oxide, copper oxide, and others) using cow urine, cow dung, and vermiwash as natural reducing and stabilizing agents. The nanoparticles produced through these environmentally friendly biological methods are characterized using techniques like UV-Vis, XRD, and SEM, and are shown to have diverse applications, including antimicrobial, antioxidant, anticancer, photocatalytic, and agricultural properties. The approach offers a sustainable, cost-effective alternative to conventional chemical synthesis methods while reducing toxic waste (P. P. Patil et al. 2025).

The synthesis of silver oxide nanoparticles using gomutra (cow urine) as a reducing agent through a combustion method at 500°C. The resulting nanoparticles, approximately 18 nm in diameter with a face-centered cubic structure, were characterized using XRD, FTIR, and FESEM techniques, and were found to contain 37.99% silver and 36.64% oxygen. The synthesized nanoparticles demonstrated significant antioxidant activity with enhanced radical scavenging capacity, suggesting potential biomedical applications as a cost-effective and environmentally friendly alternative to conventional synthesis methods (G. Allaka et al. 2023).

2.3 Anti-cancer activity of cow urine:

Cancer remains one of the leading causes of mortality worldwide, characterized by uncontrolled cell proliferation and the ability of malignant cells to invade surrounding tissues and metastasize to distant organs. Conventional cancer therapies such as chemotherapy, radiotherapy, and surgery are often associated with severe side effects,

high costs, and, in some cases, limited effectiveness. This has led to increased scientific interest in alternative and complementary therapeutic approaches, particularly those derived from natural products with fewer adverse effects.

Cow urine, known as "Gomutra" in traditional Indian medicine (Ayurveda), has been used for centuries for its therapeutic and medicinal properties. It is considered a natural bioenhancer, possessing a rich composition of volatile fatty acids, phenols, minerals, enzymes, urea, uric acid, and various bioactive compounds. Recent studies have shown that cow urine exhibits immunomodulatory, antioxidant, antimicrobial, and antineoplastic activities. The anticancer activity of cow urine has gained attention due to its ability to enhance the efficacy of conventional drugs, induce apoptosis in cancer cells, and inhibit tumor progression.

Cow urine distillate (CUD) has long been used successfully for cancer therapy, as observed in cancer patients. It also has cancer-curing potential in its advanced phases. Its beneficial properties have attracted international attention as a result of patents obtained for its anticancer effects. More time and research are still required to explain the mechanism of action and validation (I Kaur, et.al.,2022).

CUD showed to enhance the antimicrobial activity of rifampicin with a 20 µl concentration by well well-puncture method; penicillin with increasing concentration of up to 80 µl and ciprofloxacin up to 80 µl, respectively, by the disc diffusion method. The rate of degeneration of breast cancer cell lines (MCF-7) was increased with increasing concentration of CUD. Clastogen (MnO_2) of 10 µl with 200 µl of RCUD showed effective anticlastogenic activity in agarose gel electrophoresis, as the activity

of clastogen decreased with increasing concentration of RCUD. CUD acts as a bioenhancer to increase antimicrobial and antiproliferative activity. RCUD showed a high level of anticlastogenic activity toward clastogens. Thus, cow urine is found to have special properties that can be used in combination with different therapeutic agents to cure several diseases such as tuberculosis, leprosy, and cancer. Further in vivo and clinical studies are required to confirm its therapeutic efficacy (S.K MOHANVEL, et.al,2017).

Cow urine therapy is an ancient Ayurvedic practice that utilises the bioactive components of cow urine for treating various ailments. Phytochemicals from algae and medicinal plants play an important role in traditional cancer research. Targeting cancer-related proteins such as c-Jun N-terminal kinases (JNKs) and Folli statin through molecular docking provides insight into potential anticancer compounds. In silico studies using phytochemicals from cow urine extracts of red algae showed that Cyclopentane propanoic acid and Benzyl-malonic acid exhibited good binding affinities with JNKs and Folli statin, indicating their potential as anticancer agents. This suggests that cow urine-derived phytochemicals may serve as promising candidates for developing novel cancer therapies (K. Shanka, et. al., 2013).

The bio-enhancement effect of cow urine distillate has previously been demonstrated mainly with antibiotics. For the first time, our research explored this effect using various cancer cell line models, including A549, Hep-G2, MCF-7, Jurkat, and K562 cells, to study the anticancer activity of Curcumin. The results showed that cow urine– extracted Curcumin exhibited more than 100% inhibition against all tested cancer cell lines, compared to nearly 70% inhibition by pure Curcumin. This study highlights the

The potential of cow urine as a bio-enhancer in cancer therapy and opens new avenues in green chemistry for the utilisation of animal resources in human health and medical applications (H.K Manikyam et.al.,2017).

Curcumin exhibited more than 100% inhibition against all tested cancer cell lines, compared to nearly 70% inhibition by pure Curcumin. This study highlights the potential of cow urine as a bio-enhancer in cancer therapy and opens new avenues in green chemistry for the utilisation of animal resources in human health and medical applications (H.K Manikyam et.al.,2017).

Honey processed with cow urine exerts antiproliferative action on the CaCo2 cell line compared to the breast cancer cell line, which corresponds with increased phenolic content after processing. Honey processed wit cow urine exerts antiproliferative action on CaCo2 cell line compared to breast cancer cell line, which corresponds with increased phenolic content after processing (DS Swathi, et.al., 2025).

2.4 Effect of cow urine distillate as plant growth Enhancer:

The milk, cow dung, urine of the cow is used for various purposes. The present study aimed at using cow urine as a plant growth enhancer and antimicrobial agent. The plants chosen for this study were Zea mays (maize), Triticum aestivum (wheat). Cymbopogon citratum (grass), Ocimum basilicum (tukmaria) and Trigonella foenum graecum

(methi). The plants were grown for 30 days using different cow urine concentrations i. e 0% (control), 5%, 15%, 25%. The various parameters such as plant height, shoot, and root length, number of leaves, the mass of the root ete were observed. The antibacterial test using different cow urine concentrations i. e 0% (control), 5%, 15%, 25% was conducted on Escherichia coli using disc diffusion method. The fungus was screened and isolated from raw coconut and was grown on YPD media to obtain the mother culture. The media was poisoned using different cow urine concentrations, the fungi were i.e 0% (control), 5%, 15%, 25% and the fungi culture was inoculated. GCMS analysis was conducted to identify the compounds present in the cow urine. Among the concentrations, 5% cow urine concentration showed maximum growth when compared to other concentrations whereas 25% concentration showed more antibacterial and. antifungal activity when compared to others. In GCMS Analysis, 16 compounds have been identified, in which, 6 compounds were antifungal, 3 compounds were antibacterial, and 2 compounds as plant growth enhancers. This study concludes that cow urine can be used as a plant growth enhancer and it possesses antimicrobial characteristics (Sampadha Joshi et.al., 2019).

Cow urine is placed in a unique position in Indian system of medicine. In the present investigation, antifungal activity against three test fungi namely *Aspergil lus Niger*, *Aspergillus oryzae* and *Mucor* sp. which cause opportunistic mycotic infections. The antifungal activity was assessed using Poison food technique and Spore germination inhibitory activity. Considerable reduction in fungal growth, in terms of reduced colony diameter when compared to control, was observed in trials. Extent of sporulation was also reduced in plates poisoned with Cow urine distillate. The percentage of germination of spores and germ tube length was also reduced significantly. The activity of Cow urine

distillate against test fungi was found to be concentration (dose) dependent i.e., higher activity was observed when concentration is increased. The results show that Cow urine distillate possesses active principles responsible for antifungal activity and the use of Cow urine distillate could confer protection against opportunistic mycotic infections (T.R. Prashith Kekuda, et.al., 2008).

Soil-borne phytopathogen Sclerotium oryzae significantly affects rice production. To reduce the load of chemical pesticides, antifungal activity of plant extracts and cow urine against mycelial growth of *Soryzae* was tested using the poisoned food technique under in vitro conditions. Plant extracts of 2.5%, 5.0%, 7.5% and 10% concentration were prepared from *Allium cepa*, *Azadirachta indica, A. sativum*, *Ricinus communis* and *Syzygium cumini*. Inhibition of mycelial growth of *Soryzae* was recorded only in the case of *A. sativum* and *A. cepa,* while *Azadirachta indica*, *Ricinus communis* and *Syzygium cumini* did not show any inhibition of mycelial growth as compared to the control. *A. sativum* plant extracts showed maximum inhibition of mycelia growth of 68.88% at a concentration of 10% followed by 32.96%, 22.96% and 18.88% at concentrations of 7.5%, 5.0% and 2.5% respectively. 22.60%, 19.62%, 17.77% and 8.88% inhibition of mycelial growth as compared to control was recorded at 10%, 7.5%, 5.0% and 2.5% concentration of plant extracts of *A.cepa*. The concentration of cow urine inhibited the mycelial growth of S. oryzae. Cow urine at the concentrations 5, 7.5 and 10.0 per cent resulted in 100 per cent inhibition of mycelia growth of the test pathogen as compared to the control. Maximum inhibition of 98.14 per cent was observed at 2.5 per cent concentration. followed by 1.25 per cent (63.7%) concentration. This study showed that *A.sativum,* and *A.cepa,* and cow urine possess antifungal activity under

in vitro conditions. It can also be tested for antifungal activity under in vivo conditions. Keywords: Cow urine. Plant extracts, *Sclerotium oryzae* (N. Prakash et.al., 2017).

CUD showed to enhance the antimicrobial activity of rifampicin with 20 µl concentration by well well-puncture method; penicillin with increasing concentration of up to 80 µl and ciprofloxacin up to 80 µl, respectively, by the disc diffusion method. The rate of degeneration of breast cancer cell lines. (MCF-7) was increased with increasing concentration of CUD. Clastogen (Mn) of 10 µL with 200 pl of RCUD showed effective anticlastogenic activity in agarose gel electrophoresis as the activity of clastogen decreased with increasing concentration of RCUD. CUD acts as a bioenhancer to increase antimicrobial and antiproliferative activity. RCUD showed a high level of anticlastogenic activity toward clastogens. Thus, cow urine is found to have special properties that can be used in combination with different therapeutic agents to cure several diseases such as tuberculosis, leprosy, and cancer. Further in vivo and clinical studies are required to confirm its therapeutic efficacy (S. K. Mhanvel et.al.,2017).

In the present study, the antibacterial and antifungal potentials of cow urine were investigated. A total of 9 pathogenic and non-pathogenic bacterial and fungal cultures were used as test organisms against three different cow urine samples. Fungal culture includes *Aspergillus sp. Rhizopus sp. Mucor sp. Penicillium sp. Alternaria sp. Macrophomina sp* and bacterial culture include *Bacillus subtilis, Pseudomonas sp. Streptococcus sp.* The highest zone of inhibition was shown against *Aspergillus sp,*

while the smallest zone of inhibition was shown against *Macrophomina sp i*n fresh cow urine. The highest zone of inhibition was shown against *Aspergillus sp,* while the smallest zone of inhibition was shown against *Pseudomonas sp* in photoactivated cow urine. The highest zone of inhibition was shown against *Bacillus subtilis,* while the smallest zone of inhibition was shown against *Pseudomonas sp* in sterile cow urine. Based on the cumulative effect against the test organism, the raw urine sample was found to be the most efficient in inhibiting all 9 test cultures, but the activity was reported to be low against fungi compared to bacteria (T. Ghosh et.al., 2018).

An increase in resistance of *Landdu* species to routinely used antifungal agents has necessitated the quest for new drugs. Few studies have revealed that a cow's urine can suppress the growth of pathogenic fungi. Cow's urine distillate has concentrationdependent inhibitory effect on *Candida* species and is effective on the isolates that are ether resistant or sensitive to the routinely used antifungal agents (J. M. Hoh et. al., 2017).

The milk, curd, ghee, urine, and dung of the cow are considered to be sacred, as they possess some medicinal properties. The use of cow urine in fields confers resistance to plants against pathogens and pests. To give a scientific basis, we collected cow urine from the Indian breed (*cowimalnad gilda*) in the early morning. The urine tested against pathogens *Fusarium oxysporum, Claviceps purpurea*, *Rhizopus oligosporus, Aspergillus oryzae* obtained from NCIM, Pune, *Curvularia spp*, Alternaria *helianth*i and *Cladosporium spp* were collected from the infected plant parts, and the activity was tested against *Penicillium notatum* and *Trichoderma viridae.* Poison food technique to check the antifungal effect of cow urine. Considerable reduction in colony diameter and

extent of sporulation was recorded on Sabouraud's agar plates. The number of spores germinated in the presence of cow urine was also less when compared to the control. The experiment has given another observation that the older cow urine has more efficiency than the freshly collected urine samples, as it induced stress to the organism to produce secondary metabolites, which are antimicrobial (H.S. Ravikumar Patil et.al., 2007).

Considering the economic importance of the pests and to reduce the poisonous effects of pesticides to pollinators and natural enemies, studies were carried out on the efficacy of cow urine for the management of honeybee diseases, insect pests and plant diseases as an alternative to synthetic pesticides. A rapid recovery was observed in disease incidence and mite infestation in honeybee colonies of Apis mellifera during the experiments conducted at different locations of Uttarakhand, India. This novel approach of spraying cow urine for honey bee disease management has been adopted by several beekeepers of Uttarakhand. Similarly, cow urine was also found very promising against *mustard aphid*, *Lipaphis erysimi* and wheat aphids, *Microsiphum miscanthi* and *Rhopaloxiphum padi* and showed higher attractancy of natural enemies and pollinators (in mustard crop) with an increase in grain yield. On the other hand, a miraculous effect of cow urine was observed in vitro conditions against the stem root of mustard caused by *Sclerotinia sclerotiorum* with cent-per cent inhibition in mycelial growth. Cow urine was also found to be effective against disease incidence of white rust and Alternaria blight in mustard under field conditions (T. Ruchira et.al., 2016).

The present study was conducted to determine the antifungal activity of different concentrations (20%, 40%, 50%, 70% & 100%) of cow urine against some fungal pathogens (*Aspergillus flavus, Aspergillus niger, Rhizopus sp., Alternaria sp., Mucor sp., Fusarium sp., Penicillium sp., Macrophomina sp*) isolated from infected plants of Wheat. The extent of growth of test fungi in plates poisoned with cow urine was less when compared with the control plates. Among these concentrations, cow urine at 100% concentration was most effective. the cow urine has antifungal activities, and the inhibitory activity can be used in the control of fungi. The nutritional effect of cow urine on plant growth was also tested with Golden wheat (*Triticum aestivum*) (T. Ghosh et.al., 2018).

A field experiment was conducted to assess the effect of varied levels of nitrogen and cow. urine on rice crop during kharif season of 2009, laid out under a randomized block design having three replications with the six treatment combinations as T, NPK (120:60:60 kg ha), T, NPK (120:60:60 kg ha + cow urine), T, NPK (100:60:60 kg ha + cow urine), TNPK (90:60:60 kg ha + cow urine) and T = NPK (60:60:60 kg ha cow urine), including control (T). The application of nitrogen @ 90 kg ha with 60 kg ha potassium and phosphorus + cow urine (T) was found to be the best treatment regarding growth, yield and nitrogen content of paddy (M.K. Singh et.al., 2014).

2.5 Anti-Viral activity of cow urine:

Several studies have demonstrated that cow urine and its distillate exhibit significant bioactivity against various microbial pathogens, including bacteria, fungi, and viruses. The antiviral potential of cow urine has been attributed to its ability to inhibit viral replication, enhance host immune responses, and act synergistically with other therapeutic agents. Cow urine distillate has been reported to enhance the efficacy of certain antiviral compounds by improving their transport across biological membranes and increasing their bioavailability.

Treatment of CHIKV with CUD resulted in a 90% reduction in plaques with 2% v/v of CUD (EC50 ~ 0.423%). Furthermore, four of the compounds identified from GC-MS demonstrated substantial inhibition at non-toxic doses, as validated by FRET assay and Chikungunya virus plaque-reduction assay. The combination of 20 Mm TQ + 20 µM PIP + 2% v/v GA was found to be very effective against Chikungunya virus, which exhibited ~4 log or 99.99% virus reduction (R. Kumar, et.al., 2025).

The results showed that raw urine was found to be cytotoxic at the minimum concentration used, and CUD of Pahari pregnant urine were found to be effective. Extracts of both the Pahari pregnant and Pahari non-pregnant urine were found to be ineffective against Canine Parvovirus. Pahari pregnant urine distillates (CUD) were found to have mild antiviral effects against Canine Parvovirus, as they reduce some virus titre, and further studies need to be done for their substantial efficacy (H. Ravi, et.al., 2025).

2.6 Cow urine antidiabetic activity:

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is a major global health concern, associated with serious complications such as neuropathy, nephropathy, retinopathy, and cardiovascular diseases. Due to the limitations and side effects of conventional antidiabetic drugs, there is increasing interest in exploring natural, cost-effective, and safe alternative therapies.

The antidiabetic activity of cow urine is mainly attributed to its ability to improve glucose metabolism, stimulate insulin secretion, and enhance the activity of antioxidant enzymes. Bioactive components such as volatile fatty acids, phenolic compounds, nitrogenous substances, and essential minerals are believed to play a role in modulating pancreatic β-cell function and improving peripheral glucose utilization. Some studies have demonstrated that cow urine distillate, when administered to diabetic animal models, leads to a significant reduction in blood glucose levels, improved lipid profile, and better glycemic control compared to untreated groups.

The consequence of cow urine formulation (Gomutra ark, GoA) on experimental alloxan-induced diabetes in rats was studied. Wistar albino rats of either sex weighing 200-250 g were used. The biochemical parameters observed were blood sugar, vitamin C and malondialdehyde (MDA) release. GoA symptomatically drops blood glucose in diabetic rats, although the observed consequence was found to be less than standard antidiabetic, glibenclamide. The cow urine distillate also diminishes serum cholesterol and serum triglyceride levels, laterally with serum glucose level. GoA comprises

volatile fatty acids like acetic acid 2-propenyl ester, acetic acid methyl ester, 2,2,3-trichloro propionic acid, Butanoic acid-3-methyl, propyl ester, 1H-indol-3-acetate, acetic acid phenyl ester, quinoline, which act as an antioxidant and contribute to the antihyperglycemic effect (S. P. Mahajan et.al., 2020).

An herbal preparation prepared by the traditional healers of Mandsaur using cow urine and *Gymnema sylvestre* R. Br. (Asclepiadaceae), *Momordica charantia* L. (*Cucurbitaceae*), *Eugenia jambolana Lam. (Myrtaceae), Ae-gle marmelos Correa (Rutaceae), Cinnamomum tamala Buch. -Ham. (Lauraceae), Aloe barbadensis Linn. (Liliaceae)* and *Trigonella foenum-graecum* L. (*Leguminosae*) are being used in the treatment of diabetes. In order to scientifically appraise the claim, this preparation was studied for antidiabetic activity and also compared with the herbal preparation prepared using water. Fresh cow urine was also used in the study to identify the synergistic effect. The preparations were tested for antidiabetic activity in alloxan-induced diabetic rats at two dose level, 200 and 400 mg/kg, respectively. The study was done for a period of 21 days. The activity was compared with reference standard, insulin (1 unit/kg, i.p.) and control. The herbal preparations significantly ($P<0.05$, $P<0.01$) lowered the blood sugar level of hyperglycemic rats in a dose-dependent manner. Comparatively, the cow urine preparation showed better activity than did the preparation prepared using water.

Fresh cow urine also exhibited significant antidiabetic effect. This study supports the claim of the local traditional healers (E.E. Jarald et.al., 2009).

2.7 Effect of cow urine as an antioxidant:

Oxidative stress is a major factor involved in the pathogenesis of numerous diseases, including cancer, diabetes, cardiovascular disorders, neurodegenerative diseases, and ageing. It results from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense mechanisms. Antioxidants are crucial for neutralizing ROS, thereby preventing cellular damage and maintaining physiological homeostasis.

Several studies have demonstrated that cow urine exhibits strong antioxidant activity, as evaluated by standard in vitro assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) assay, Ferric Reducing Antioxidant Power (FRAP) assay, and Hydrogen Peroxide scavenging assay. The ability of cow urine to reduce DPPH radicals indicates the presence of electron-donating substances, while FRAP values correlate with its reducing power and overall antioxidant capacity.

The antioxidant property of cow urine plays an important role in its reported immunomodulatory, antimicrobial, antidiabetic, anticancer, and hepatoprotective activities, as these effects are often linked to the reduction of oxidative damage in biological systems. In addition, cow urine has been found to synergize with other herbal compounds or antibiotics, possibly through the enhancement of cellular antioxidant def An herbal preparation prepared by the traditional healers of Mandsaur using cow urine and *Gymnema sylvestre* R. Br. (Asclepiadaceae), *Momordica charantia L.* (Cucurbitaceae), *Eugenia jambolana Lam.* (Myrtaceae), *Ile-gle marmelos Correa* (Rutaceae), *Cinnamomum tamala Buch. -Ham.* (Lauraceae), *Aloe barbadensis*

(Liliaceae), and *Trigonella foenum-graecum L.* (Leguminosae) are being used in the treatment of diabetes. In order to scientifically appraise the claim, this preparation was studied for antidiabetic activity and also compared with the herbal preparation prepared using water. Fresh cow urine was also used in the study to identify the synergistic effect. The preparations were tested for antidiabetic activity in alloxan-induced diabetic rats at two dose levels, 200 and 400 mg/kg, respectively. The study was done for a period of 21 days. The activity was compared with the reference standard, insulin (1 unit/kg, i.p.) and control. The herbal preparations significantly ($P<0.05$, $P<0.01$) lowered the blood sugar level of hyperglycemic rats in a dose-dependent manner. Comparatively, the cow urine preparation showed better activity than the preparation prepared using water. Fresh cow urine also exhibited a significant antidiabetic effect. This study supports the claim of the local traditional healers.

Cow urine is considered to possess immense therapeutic properties. The aim of the study was to evaluate the biochemical, antioxidant and antimicrobial potential of cow urine. We have used paper chromatography, DPPH antioxidant assay as well as disc diffusion assay to establish the presence of amino acids in the urine and also to portray its antimicrobial and antioxidant potential. We have been able to identify arginine, glutamine and serine in the cow urine. Cow urine proved to be an effective antibacterial agent as depicted by the zone of inhibition in disc diffusion assay. DPPH assay confirms cow urine as a potent antioxidant. The study concludes that cow urine can be used as an effective antimicrobial as well as antioxidant (A.sharma , et.al 2019).

The number of plants and animal-derived materials was reported to have antioxidant and antimicrobial activity. The present study relates to such precious and holy animal derived material, cow urine, which has these activities. Antioxidant activity was measured using two in vitro models, DPPH radical scavenging activity and Superoxide scavenging activity. Ascorbic acid was used as the reference standard. The antimicrobial activity of cow urine and its distillate was tested by agar well method using the microbes like *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermitis*, *Bacillus subtilis*, *Klebsiella p* urine and its distillate tested for antioxidant and antimicrobial activities exhibited the mentioned activities, and comparatively fresh cow urine was found to be better than its distillate. These results indicate that the cow urine has antioxidant and antimicrobial activities, which support the claim of traditional practitioners (E. Jarald et.al., 2008).

Cow urine occupies a holy place in Indian rituals and Ayurveda due to its effective application against various diseases since the Vedic period. The present study was carried out to evaluate the antibacterial and antioxidant properties of Badri cow urine. The antibacterial potential was evaluated against *Staphylococcus aureus. Listeria monocytogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella typhi* and minimum inhibitory concentration (MIC) evaluated following different methods. The total phenolic contents, free radical and superoxide radical scavenging activity were assessed by FC, DPPH and NBT methods. Cow urine inhibited the growth of all selected bacterial pathogens with different potencies; however, *S. aureus, L. monocytogenes and E. coli* were the most susceptible bacterial strains to cow urine even at low concentrations. The total phenolic content of cow urine was 5.09 mg GAE/g. The DPPH and NBT assays showed dose-dependent scavenging activity of cow

urine. Due to the presence of both antibacterial and antioxidant properties, urine may be used as a therapeutic agent (R.C. Dubey 2020).

Objectives

1) To collect cow urine and to obtain cow urine distillate (CUD)
2) To prepare different agar media using CUD
3) To prepare different broth media using CUD as a base
4) To check the efficiency of CUD agar and broth for microbial growth and other characteristics

3.0 Materials and Methods

3.1 Collection of Cow Urine

Fresh cow urine was collected from a healthy indigenous cow (Gir breed) from a local farm in the village area of Murgud. The sample was collected early in the morning in a sterile glass container (1 L capacity) to avoid contamination. The collected urine sample was immediately transported to the laboratory under hygienic conditions. The sample was filtered through sterile Whatman No. 1 filter paper to remove dust particles, debris, and other impurities. After filtration, the urine sample was transferred into a sterile, labelled glass flask and stored at 4°C in a refrigerator until further experimental use. All procedures were carried out under aseptic conditions to prevent microbial contamination of the sample.

3.2 Preparation of cow urine distillate (CUD)

The filtered cow urine sample was used to prepare cow urine distillate (CUD) by distillation. Initially, the filtered cow urine was transferred into a distillation flask of a distillation apparatus. The flask was then heated gently using a heating mantle. During heating, vapours of volatile components present in the cow urine were produced. These vapours passed through the condenser, where they were cooled and condensed into liquid form. The condensed liquid obtained after the distillation process was collected in a sterile glass container. The collected distillate, known as Cow Urine Distillate (CUD), was properly labelled and stored at 4°C in a refrigerator until further analysis and experimental use. All the procedures were carried out under aseptic laboratory conditions to avoid contamination

3.3 Estimation of ammonia from cow urine distillate.

3.3.1 Preparation of Nessler's reagent

Nessler's reagent was prepared according to the standard laboratory procedure. Initially, 10 g of mercuric chloride ($HgCl_2$) was dissolved in 60 ml of distilled water to prepare the first solution. In a separate container, 7 g of potassium iodide (KI) was dissolved in 20 ml of distilled water. Both solutions were then mixed slowly with continuous stirring, resulting in the formation of a red precipitate of mercuric iodide (HgI_2). Separately, 16 g of potassium hydroxide (KOH) was dissolved in 20 ml of distilled water. The prepared KOH solution was then added slowly to the above mixture with constant stirring. Finally, the solution was made up to 100 ml with distilled water. The prepared reagent was filtered if necessary and stored in a clean reagent bottle for further use.

3.3.2 Preparation of standard stock solution

Weighed 3.819 g of ammonium chloride (NH4CL). Dissolved in distilled water. Made the final volume up to 1 litre. The stock standard solution was 1000ppm.

3.3.3 Preparation of working standard solution

A working standard solution was prepared by diluting the stock standard solution with distilled water. A working standard solution was prepared at 1 ppm to 10 ppm.

3.3.4 Preparation of blank

Blank solution contains 5ml distilled water, 0.5 ml Nessler's reagent.

3.3.5 Sample preparation

Took a 5ml CUD sample in a test tube. Added 0.5ml Nessler's reagent and mixed gently.

3.3.6 Preparation of standard graph

A series of calibration standards was prepared to construct a standard curve for ammonia estimation using Nessler reagent (Table 1). Ten clean and dry test tubes were taken and labelled from 1 to 10. Different volumes of the standard ammonia solution were pipetted into each tube. Distilled water was added to make the total volume 10 ml in each tube.

Table 1: Preparation of the standard graph

Tube	Standard ammonia solution (ml)	Distilled water (ml)	Nessler's reagent (ml)	Incubation at room temperature
1	1	9	0.5	15min
2	2	8	0.5	15min
3	3	7	0.5	15min
4	4	6	0.5	15min
5	5	5	0.5	15min
6	6	4	0.5	15min
7	7	3	0.5	15min
8	8	2	0.5	15min
9	9	1	0.5	15min
10	10	0	0.5	15min
11 (Sample)	10 (CUD)	0	0.5	15min

3.3.7 Color development

After the addition of Nessler reagent, the mixture was gently mixed and allowed to stand for about 15 minutes at room temperature. A yellow to brown color developed depending on the concentration of ammonia present in the sample. The intensity of the color indicated the amount of ammonia formed.

3.3.8 Detection of ammonia

The spectrophotometer was set to 420 nm. Blank solution was used to calibrate the instrument. Absorbance was measured for all standard solutions and the sample solution.

3.3.9 Preparation of standard graph

A graph was plotted between ammonia concentration (ppm) on the x-axis. Absorbance at 420nm on the y-axis. A linear standard curve will be obtained.

3.3.10 Quantitative estimation of ammonia

Absorbance of the CUD sample was noted. The corresponding concentration on the standard curve was located. Ammonia concentration in the sample was calculated.

3.4 Preparation of CUD-based agar medium

4.6.1Preparing different dilutions

Different dilutions of Cow Urine Distillate (CUD) were prepared using sterile distilled water, as shown in Table 2.

Table 2 Dilutions of CUD

Dilutions	CUD (ML)	Distilled water (ml)
1:10	10	90
1:20	5	95
1:30	3.3	96.7
1:40	2.5	97.5

3.4.2 CUD agar media preparation

The required dilution of CUD was taken in a conical flask. The pH of the medium was adjusted to **7.0**. Agar powder (15–20 g/L) was added to the solution. The medium was sterilized by autoclaving at 121°C for 15 minutes. Sterilized CUD agar medium was cooled to 40 to 30°C. Approximately 20 ml of medium was poured into sterile petri plates. Plates were allowed to solidify under aseptic conditions.

3.4.3 Inoculation of the organisms

A pure culture of microorganisms was obtained from a laboratory stock culture. A sterile inoculating loop was used to spot-inoculate the organisms like *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Enterobacter hormaechi, Klebsiella variicola* and *Azotobacter chroococcum,* and *Candida albicans* on CUD media. Plates were incubated at 37°C for 24 hours.

3.5 Preparation of CUD MacConkey's agar as a selective agar medium

3.5.1 Preparation of alternative media for MacConkey's agar using CUD

About 50 ml of CUD dilution (1:40) was taken. Lactose powder, bile salts and neutral red indicator were added to the medium. Agar-agar powder was added. The medium was sterilised in an autoclave. Sterile medium was poured into Petri plates. *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Enterobacter hormaechi, and Klebsiella variicola* were inoculated, and the plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for pink colonies.

Table 3 CUD-based MacConkey's agar

Sr. no.	Component	Quantity
1	Cow Urine Distillate (CUD)	1.25 ml
2	Lactose	0.5 gm
3	Bile salts	0.25 gm
4	Neutral red	Trace amount
5	Agar-agar	1.75 gm
6	Distilled water	48.75 ml

3.5.2 Preparation of CUD Starch agar medium

About 50 ml of CUD dilution (1:40) was taken. Starch powder and agar powder were added to the CUD dilution. The medium was sterilised by autoclaving. Sterile medium was poured into Petri plates. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Enterobacter hormaechi, Klebsiella variicola* and *Azotobacter chroococcum*, and *Candida albicans* were inoculated, and plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for a starch hydrolysis zone.

Table 4 CUD-based starch agar

Sr. No.	Components	Quantity
1	CUD	1.25 ml
2	Starch powder	0.5 gm
3	Agar agar powder	1.75 gm
4	Distilled water	48.75 ml

3.5.3 Preparation of CUD Milk agar medium

1: 40 CUD dilution was taken. Agar powder was added and sterilised in an autoclave. Sterile milk (5 ml) was prepared separately using a water bath. After sterilisation, sterile milk was added to the medium. The medium was poured into Petri plates. *Bacillus subtilis and Staphylococcus aureus* were spot inoculated on plates and incubated at 37°C for 24 hours. After incubation, the plates were observed for a casein hydrolysis zone.

Table 5 CUD -based milk agar

Sr. No.	Component	Quantity
1	CUD	1.25 ml
2	Milk	5 ml
3	Agar agar	1.75 gm
4	Distilled water	48.75 ml

3.5.4 Preparation of CUD Gelatin agar medium

50 ml of CUD dilution (1:40) was measured. Gelatin powder and agar powder were added to the medium. The medium was sterilised in an autoclave. Sterile medium was poured into Petri plates. *Bacillus subtilis* was inoculated, and plates were incubated at

37°C for 24 hours. After incubation, plates were observed for gelatin hydrolysis. Frazier's reagent was added to detect gelatin hydrolysis.

Table 6 CUD-based gelatin agar

Sr. No.	Component	Quantity
1	CUD	1.25ml
2	Gelatin powder	0.5gm
3	Agar agar	1.75gm
4	Distilled water	48.75gm

3.5.5 Preparation of CUD Tween 80 agar medium

50 ml of CUD dilution (1:40) was taken, and agar powder was added. The medium was sterilised by autoclaving. Tween 80 was added to the sterile medium. The medium was poured into Petri plates. *Bacillus subtilis* was inoculated, and plates were incubated at 37°C for 24 hours. After incubation, the plates were observed for a degradation zone of Tween 80.

Table 7 CUD-based tween 80 agar

Sr. No	Component	Quantity
1	CUD	1.25 ml
2	Tween 80	5ml
3	Agar agar	1.75gm
4	Distilled water	48.75gm

3.5.6 Preparation of CUD Phenylalanine deamination media

Cow Urine Distillate (CUD) dilution (1:40) was prepared and mixed with agar– agar powder to prepare the basal medium. Phenylalanine powder was added to the medium. The medium was sterilised by autoclaving at 121°C for 15 minutes. After sterilisation, the medium was poured into sterile test tubes to prepare blanks. The *Bacillus subtilis* was inoculated into the tubes using aseptic techniques. The inoculated tubes were incubated at 37°C for 24 hours.

Table 8 CUD-based phenylalanine agar

Sr. No.	Component	Quantity
1	CUD	1.25ml
2	Phenylalanine	0.5gm
3	Agar agar	1.75gm
4	Distilled water	48.75ml

3.6 Preparation of CUD-based broth medium

3.6.1 Preparation of an alternative broth medium for nutrient broth by using CUD

Cow urine distillate, 1:40 dilution, was prepared (50 ml), and it was sterilised by autoclaving. After cooling down, it was poured into sterile test tubes and inoculated with *Enterobacter hormaechi, Klebsiella variicola and Azotobacter chrococcum* and was kept for incubation at room temperature. After incubation, the tubes were observed for turbidity.

3.6.2 Preparation of CUD amino acid decarboxylation medium

CUD dilution (1:40) was taken in two separate flasks. Glucose (0.2%) and bromocresol purple indicator were added to the medium. Amino acids were added separately. Lysine was added to one flask. Arginine was added to the second flask. The media were distributed in test tubes. The tubes were sterilised by autoclaving. The *Bacillus subtilis* was inoculated into the tubes. The tubes were incubated at 37°C for 24 hours.

Table 9 CUD-based amino acid decarboxylation medium

Sr. No	Composition	Quantity
1	CUD	1.25ml
2	Glucose (0.2%)	0.1gm
3	Bromocresol Purple	0.02gm
4	Lysine (for Flask 1)	0.5gm
5	Arginine (for Flask 2)	0.5gm
6	Distilled water	48.75ml

3.6.3 Preparation of CUD sugar fermentation media

CUD dilution (1:40) was taken in 5 different flasks. Peptone powder was added, and its concentration was made 1%. Sugars such as Glucose, Sucrose, Lactose, Maltose and Galactose were added to make the final concentration 1%. Andrade's indicator was added to each flask. These media were sterilised by autoclaving and poured into different tubes, including a Durham tube. Organisms such as *Escherichia coli* and *Staphylococcus aureus* were inoculated, and the tubes were kept for incubation at 37°C for 24 hours.

Table 10 CUD- based sugar fermentation media

Sr. No.	Components	Quantity
1	Cow Urine Distillate	1.25ml
2	Peptone (1%)	0.5gm
3	Andrade's indicator	2 to 3 drops
4	Glucose (1%) – Flask 1	0.5gm
5	Sucrose (1%) – Flask 2	0.5gm
6	Lactose (1%) – Flask 3	0.5gm
7	Maltose (1%) – Flask 4	0.5gm
8	Galactose (1%) – Flask 5	0.5gm
9	Distilled water	48.75ml

3.7 CUD agar costing

The costing of different microbiological media was carried out by calculating the price of raw materials and operational expenses required for the preparation of 100 ml medium. The market price of commercially available media was first recorded. Nutrient Agar (NA) of 500 g was priced at ₹4047, MacConkey Agar (MAC) of 500 g at ₹4549, and Potato Dextrose Agar (PDA) of 500 g at ₹6591. The cost per 100ml of each medium was calculated by dividing the total cost by the total weight.

4.0 Result and Discussion

4.1 Collection of cow urine

Cow urine was collected in a clean, sterile container directly from a healthy cow as shown in the Fig. No. 1.

Fig No 1: Sample of raw cow urine

4.1 Preparation of cow urine distillate

Preparation of cow urine distillate involves collecting fresh cow urine and subjecting it to distillation to obtain a purified, sterile liquid containing volatile bioactive components (Fig No. 2).

Fig No 2: Distillation process of raw cow urine

4.1 Estimation of ammonia from cow urine distillate

Ammonia concentration was estimated using Nessler's reagent method (Fig. No. 3), and absorbance was measured at 420 nm using a spectrophotometer, which is shown in Table11

Table 11 Estimation of ammonia in CUD

Ammonia concentration (ppm)	Absorbance at 420nm
1ppm	0.08
2ppm	0.20
3ppm	0.25
4ppm	0.30
5ppm	0.42
6ppm	0.45
7ppm	0.49
8ppm	0.49
9ppm	0.45
10ppm	0.44
0ppm (Blank)	0
Sample	0.15

Fig No 3: Estimation of ammonia using Nessler's reagent

When the graph of Ammonia concentration (ppm) vs. absorbance at 420 nm was plotted, it showed that absorbance increased with an increase in ammonia concentration, indicating a positive correlation between ammonia concentration and color intensity.

4.1.1 Standard graph

The calibration curve of standard ammonia solutions exhibited a linear relationship between ammonia concentration and absorbance up to approximately 7 ppm

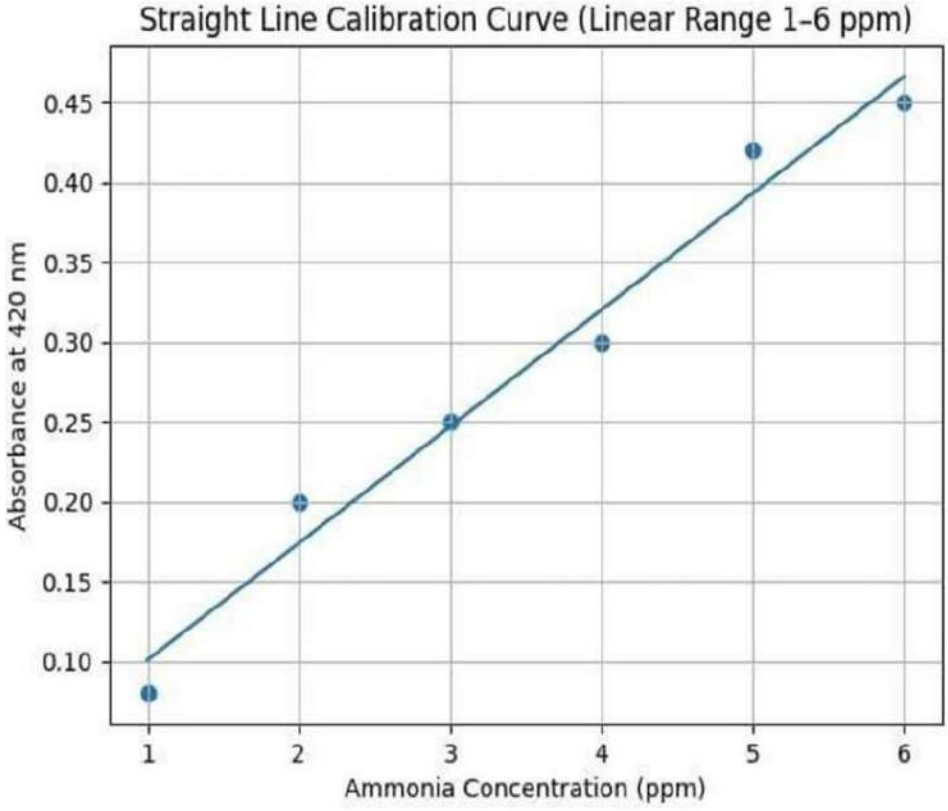

Fig no.4: Standard graph of Ammonia Concentration

Absorbance of the sample recorded at 420 nm was found to be 0.15. From the standard curve, 0.15 absorbance ≈ ~1.5 ppm ammonia. Therefore, Ammonia concentration in Cow Urine Distillate ≈ is 1.5 ppm

4.2 Evaluation of CUD-based agar medium

The results obtained from the study showed that CUD (Cow Urine Distillate) medium was able to support the growth of a wide range of microorganisms at different dilutions: 1:10, 1:20, 1:30, 1:40. (Table 12) (Fig No 5), (Fig No 6),

Table 12 Growth of organisms across different dilutions (1:10, 1:20, 1:30, and 1:40)

Organism	1:10	1:20	1:30	1:40
Escherichia coli	-	-	-	+
Staphylococcus aureus	-	-	+	++
Bacillus subtilis	++++	++++	++	++
Klebsiella pneumoniae	++++	++++	++++	++++
Proteus vulgaris	++++	++++	++++	++++
Salmonella typhi	++++	++++	++++	++++
Enterobacter hormaechi	++++	++++	++++	++++
Klebsiella variicola	++	++	+++	++
Azotobacter chrococcum	-	-	+++	+
Candida albicans	-	-	+	++

+ indicates low growth, ++ indicates medium growth, +++ indicates high growth, ++++ indicates highest growth, - Indicates no growth

Fig No. 5: Growth of organisms across 1:10 dilution.

Fig No. 6: Growth of organisms across 1:20 dilution.

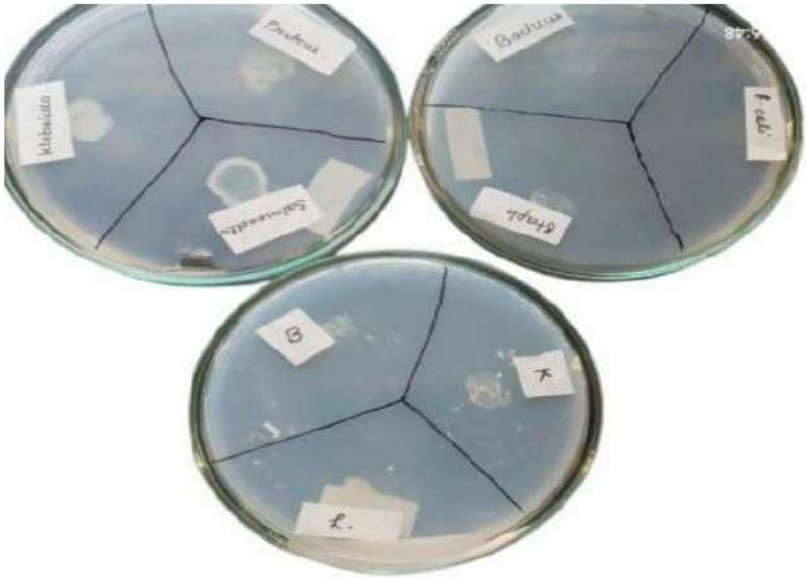

Fig No. 7: Growth of organisms across a 1:30 dilution.

Fig No. 8: Growth of organisms across a 1:40 dilution.

Pathogenic bacteria such as *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris*, and *Salmonella typhi* showed growth in Cow Urine Distillate (CUD) medium at different dilutions. Strong growth was observed, particularly for *Klebsiella pneumoniae, Proteus vulgaris* and *Salmonella typhi* across all tested dilutions (1:10, 1:20, 1:30, and 1:40) at 37°C for 24 hours, indicating that CUD contains sufficient nutrients to support the growth of these organisms. *Staphylococcus aureus* showed growth mainly at higher dilutions, suggesting that concentrated CUD may have a slight inhibitory effect. Here, pathogenic as well as beneficial organisms showed growth.

Beneficial microorganisms such as *Enterobacter hormaechi*, *Klebsiella variicola* and *Azotobacter chroococcum*, which are important in agriculture and soil fertility, showed limited growth at lower dilutions but improved growth at higher dilutions. This suggests that diluted CUD medium can support beneficial microbes and may be useful for agricultural microbiology studies.

Non-pathogenic organisms like *Enterobacter hormaechi.* showed moderate to good growth in CUD medium. This indicates that the medium can support the growth of several microorganisms. However, some organisms showed better growth only at higher dilutions. The fungal organism *Candida albicans* showed very minimal growth in the CUD medium. Growth was observed only at higher dilutions, indicating that the medium is less suitable for fungal organisms. This may be due to antifungal compounds present in cow urine. (Fig 5), (Fig 6), (Fig 7) and (Fig 8).

4.3 Evaluation of CUD MacConkey's agar as a selective agar medium

When different organisms were streaked on CUD MacConkey's agar medium, results indicated that CUD medium differentiated lactose-fermenting and non-lactose-fermenting organisms, suggesting that it served as a potential alternative to MacConkey's agar.

Table 13 CUD MacConkey's agar as a differential alternative medium for MacConkey's agar

Organism	Result of lactose-fermenting and non-lactose-fermenting organisms
Escherichia coli	Pink colony
Staphylococcus aureus	No colony
Bacillus subtilis	Pink colony
Klebsiella pneumoniae	Pink colony
Proteus vulgaris	Colorless
Salmonella typhi	Colorless
Enterobacter hormaechi	Pink colony
Klebsiella variicola.	Pink colony

Organisms such as *E. coli, Bacillus subtilis, Klebsiella pneumoniae*, and *Enterobacter hormaechi* produced pink colonies, indicating lactose fermentation and acid production in the medium. This observation was similar to the characteristic reaction seen on MacConkey's agar (Table 13) (Fig 9).

On the other hand, *Proteus vulgaris*, *Salmonella typhi*, *Klebsiella variicola*, and *Azotobacter chroococcum* produced colourless colonies, indicating the absence of lactose fermentation (Table 13) (Fig 9).

Staphylococcus aureus showed no colony formation, suggesting that the medium inhibited the growth of some Gram-positive bacteria (Table 13) (Fig 9).

Fig No. 9: Growth of different microorganisms on CUD MacConkey's agar

Fig No. 10: Growth of different microorganisms on CUD MacConkey's agar

4.4 Evaluation of CUD on Starch agar medium

The findings suggested that CUD starch medium could be used as an alternative to starch agar for detecting amylase activity (Table 14) (Fig 11).

Table 14: Activity of organism on CUD starch agar.

Organism	Result of Amylase activity
Escherichia coli	No growth
Staphylococcus aureus	No growth
Bacillus subtilis	Clear zone
Klebsiella pneumoniae	Clear zone
Proteus vulgaris	Clear zone
Salmonella typhi	Only colony
Enterobacter hormaechi	Growth
Klebsiella variicola	Growth

Bacillus subtelis, *Klebsiella pneumoniae*, and *Proteus vulgaris* showed clear zones after the addition of iodine solution, indicating the production of amylase enzyme and the hydrolysis of starch (Table 14) (Fig 11).

E. coli and *Staphylococcus aureus* showed no growth, suggesting that the medium did not support their growth under these conditions (Table 14) (Fig 11).

Other organisms such as *Salmonella, Enterobacter hormaechii, Klebsiella variicola,* and *Acinetobacter chrococcum* showed growth without clear zones after addition of iodine solution, indicating the absence of starch hydrolysis (Table 14) (Fig 11).

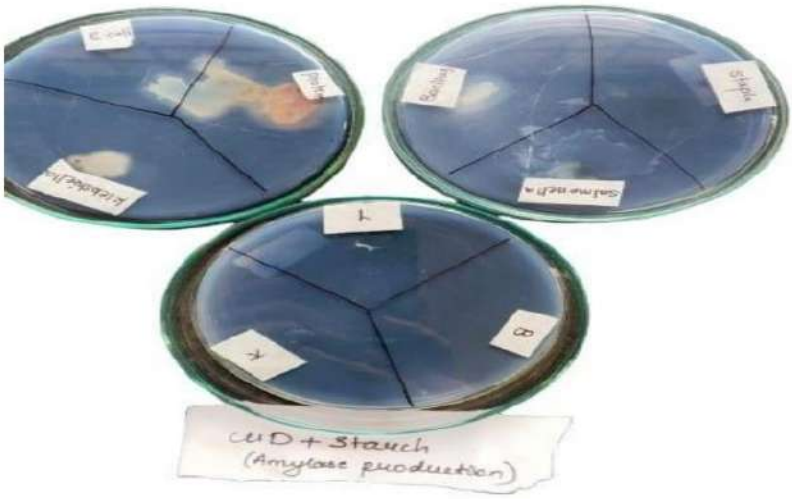

Fig No. 11: Amylase activity on CUD Starch agar medium

4.5 Evaluation of CUD Milk agar medium

These observations suggested that CUD milk agar could be used as an alternative medium for detecting casein hydrolysis (Table 15) (Fig 12).

Table 15: Activity of the organism on CUD milk agar

Organism	Result of Casein hydrolysis
Bacillus subtelis	+
Staphylococcus aureus	+

+ Indicates the growth with the zone of hydrolysis of casein.

The results showed that *Bacillus* and *Staphylococcus aureus* produced a clear zone around colonies after incubation at 37°C for 24 hours, indicating casein hydrolysis.

Fig No. 12: Activity of Casein hydrolysis on CUD Milk agar medium

4.6 Evaluation of CUD Gelatin agar medium

When the Frazier's reagent was poured on gelatin agar plate containing colonies, gelatin hydrolysis zone was observed, results indicated that CUD gelatin agar medium acted as an effective alternative to gelatin agar for detecting Gelatin hydrolysis (Table 16) (Fig 13).

Table 16: Activity of Gelatin hydrolysis on CUD Gelatin agar medium

Organism	Result of Gelatinase activity
Bacillus subtilis	+

+ Indicates Gelatin hydrolysis

Fig No. 13: Activity of Gelatin hydrolysis on CUD Gelatin agar medium

4.7 Evaluation of CUD Tween 80 agar medium

The results indicated that CUD Tween 80 agar medium acted as an effective alternative to Tween 80 agar for detecting Tween 80 hydrolysis (Table 17) (Fig 14).

Table 17: Lipase activity of organism on Tween 80 agar

Organism	Result of lipase activity
Bacillus subtilis	+

+ Indicates growth with hydrolysis of Tween 80

Fig No. 14: Activity of Lipase on CUD Tween 80 agar medium

4.8 Evaluation CUD Amino acid decarboxylation medium

The ability of CUD broth to support amino acid decarboxylation reactions was also tested. *Bacillus subtilis* showed positive results in lysine decarboxylation, while *Bacillus subtilis* also showed positive results in arginine decarboxylation. The positive result was indicated by growth and a colour change in the medium.

These results demonstrated that CUD medium could be used for detecting amino acid decarboxylation reactions (Table 18) (Fig 15) and (Table 19) (Fig 16)

Table 18: CUD Broth with Lysine

Organism	Result of lysine decarboxylation
Bacillus subtilis	+

+ Indicates growth of the organism with Lysine decarboxylation

Fig No.15: CUD Amino acid decarboxylation medium for Lysine decarboxylation

Table 19: CUD Broth with Arginine

Organism	Result of Arginine decarboxylation
Bacillus subtilis	+

+ Indicates growth of the organism with Arginine decarboxylation

Fig No.16: CUD Amino acid decarboxylation medium for Arginine decarboxylation

4.9 Evaluation of CUD Sugar fermentation medium

The results suggested that CUD medium acted as an alternative medium for studying sugar fermentation reactions (Table 20).

Table 20: CUD Sugar Fermentation Test

CUD (1:40) +Sugar	Organism	Result of sugar ferment ation
Glucose	***Escherichia coli***	+
Lactose	***Escherichia coli***	+
Galactose	***Escherichia coli***	+
Maltose	***Escherichia coli***	+
Sucrose	***Staphylococcus aureus***	+

+ Indicate growth of organisms and fermentation of sugar and acid production.

Fig No. 17: CUD sugar fermentation medium for Glucose fermentation

Fig No. 18: CUD sugar fermentation medium for Lactose fermentation

Fig No. 19: CUD sugar fermentation medium for Galactose fermentation

Fig No. 20: CUD sugar fermentation medium for Maltose fermentation

Fig No.21: CUD sugar fermentation medium for Sucrose fermentation

4.11 The total cost for preparation of CUD agar

Based on standard preparation protocols, the required quantities for 100 ml medium were taken as 2.8 g for NA, 5.5 g for MAC, and 3.9 g for PDA. Using the cost per gram, the total cost for 100 ml was calculated as approximately ₹33.98 (≈₹34) for NA, ₹61.71 for MAC, and ₹62.72 for PDA.

For the preparation of CUD-based agar medium, different dilutions of cow urine distillate (1:10, 1:20, 1:30, and 1:40) were used. Agar-agar was added as a solidifying agent, where the cost of agar was calculated as ₹11.32 per gram. For 2.5 g agar used per 100 ml, the cost was ₹28.32. Additionally, electricity consumption during preparation (approximately 2 hours) was considered, where ₹9 was required for 100 ml medium, corresponding to ₹0.09 per ml.

The total cost of CUD-based media per 100 ml was then calculated by adding agar cost and electricity charges. The final costs obtained were ₹29.22 for 1:10 dilution, ₹28.77 for 1:20 dilution, ₹28.61 for 1:30 dilution, and ₹28.54 for 1:40 dilution.

4.0 Summary and Conclusion

The present study demonstrated the potential application of Cow Urine Distillate (CUD) as an alternative and economical component for microbiological culture media. Fresh cow urine was collected from a healthy Gir breed cow and processed under aseptic conditions to obtain cow urine distillate through the distillation method.

The distillate was analysed for ammonia content using Nessler's reagent method and evaluated for its ability to support microbial growth when used in different culture media formulations. The estimation of ammonia in cow urine distillate showed that the sample contained approximately 1.5 ppm ammonia, as determined by comparing the absorbance value (0.15 at 420 nm) with the standard calibration curve. The results indicated a positive relationship between ammonia concentration and colour intensity, confirming the presence of measurable nitrogenous compounds in the distillate. These compounds may contribute to the nutritional value of the medium and support microbial growth.

The study also evaluated the ability of CUD to support microbial growth at different dilutions (1:10, 1:20, 1:30, and 1:40). The results showed that several microorganisms, including *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi,* and *Enterobacter hormaechi*, were able to grow in the CUD-based medium. Among these, organisms such as *Klebsiella pneumoniae, Proteus vulgaris, and Salmonella typhi* showed strong growth, indicating that CUD contains sufficient nutrients required for bacterial metabolism. Some organisms showed better growth

at higher dilutions, suggesting that concentrated CUD may have mild inhibitory effects due to certain antimicrobial compounds present in cow urine.

In addition to supporting bacterial growth, CUD was successfully used to prepare different alternative microbiological media such as CUD-based MacConkey agar, starch agar, milk agar, gelatin agar, Tween 80 agar, and amino acid decarboxylation media. The results obtained from these experiments indicated that CUD-based media were able to detect various microbial biochemical activities such as lactose fermentation, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, Tween 80 hydrolysis, amino acid decarboxylation, and sugar fermentation. These observations were comparable to the reactions observed in conventional microbiological media.

Furthermore, the CUD-based MacConkey agar was able to differentiate lactose-fermenting organisms (which produced pink colonies) from non-lactose-fermenting organisms (which produced colourless colonies). Similarly, CUD starch agar allowed detection of amylase activity through clear zones around colonies, while CUD milk agar and gelatin agar enabled the detection of casein and gelatin hydrolysis, respectively. These findings highlight the functional efficiency of CUD as a base for preparing different selective and differential culture media. The overall cost analysis demonstrated that CUD-based media are significantly more economical compared to commercially available media such as NA, MAC, and PDA, with decreasing cost observed at higher dilutions

Overall, the results of this study indicate that Cow Urine Distillate can serve as a cost-effective, natural, and sustainable alternative for the preparation of microbiological culture media.

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