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RESEARCH ARTICLE

A STUDY ON ANTIBACTERIAL ACTIVITY AND EXTENSION OF SHELF LIFE OF FRESH COW URINE

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Abstract

Cow urine has been used as medicine since ancient times andthe ancient scriptures of Ayurveda consider cow urine to be the elixir of life. The present study analyzes the inhibitory effects of fresh cow urine at different pH (8.2,4.4,3.8) at the intervals of 5,10, 20,30 days and antibacterial activity of cow urine at different pH was analyzed against the *Escherichia coli , Staphylococcus aureus* and *Klebsiella sp*. It was found that no bacterial growth was present in fresh cow urine at pH 3.8 after 30 days. Shelf life of cow urine could be extended after lowering the pHwhich also makes it palatable as it becomes odourless. Maximum antibacterial activity was also observed in fresh cow urine sample at pH 3.8 against gram positive and gram-negative bacteria *Escherichia coli* (24mm ± 2.0), *Staphylococcus aureus* (22mm ± 2.0) and *Klebsiella sp* (22mm ± 1.0).Fresh cow urine at pH 3.8 was found to be more active and exhibited better antibacterial activity.It can be used in the control of bacteria.

Introduction:-

In the Ayurvedic texts Charka Samhita, Sushruta Samhita and Ashtanga Sangraha, cow urine (CU) is a unique part of Ayurveda and has been taken as a medicine since ancient times. Gomutra is called ‘Sanjivani’ and ‘Amrita’ in Ayurveda. It is used in ayurvedic formulation either alone or in combination. Several medicinal properties have been reported in cow urine such as cardiac and renal diseases, indigestion, stomach ache, diarrhea, edema, jaundice, anemia, hemorrhoids and skin diseases including vitiligo.Various research papers reported the presence of nitrogen, sulphur, phosphate, sodium, manganese, iron, silicone, chlorine, magnesium, citric, malic, succinic, carbolic acid, calcium salts, vitamins A,B,C and D lactose, enzymes, creatinine hormones [1]. CU contains 95% water, 2.5% urea and 2.5% a mixture of salt hormones, enzymes and minerals. Cytokines and amino acids present in urine may play a role in immune enhancement [2]. It has been reported that cow urine increases the phagocytic activity of macrophages and is thus helpful against bacterial infections [3,4,5]. CU is basically an excellent germicide and a potent antibiotic. Therefore, cow urine therapy boosts immunity and kills all the pathogenic bacteria if consumed daily. Cow urine also acts as a bio-enhancer [6].

Cow urine has been granted US Patents (No. 6,896,907 6,410,059 and 6,410,059).Nowadays, different preparations of cow urine like cow urine distillate, photo-activated urine and fresh urine have been marketed and sold . Fresh urine was found to be more active than distillate. Fresh CU exhibited better antimicrobial activity [7]. Fresh CU contains higher amounts of phenols than cow urine distillate (CUD) makes it more effective against microbes [8]. CU is an effective antibacterial against a broad spectrum of gram positive and gram-negative bacteria. The microbial population has been characterized in CU and revealed the total bacterial population was 260 x10^4cfu/ml at alkaline

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pH [9,10]. Raw cow urine has a shelf-life of six to eight hours after which it becomes toxic due to the presence of ammonia. If the pH becomes low the microbial activity decreases and at low pH microbes are killed.Lowering its pH reduces ammonia and urea content of cow urine. Chemicals like sodium chloride, sodium nitrate and nitrate, sodium benzoate, ascorbic acid, propionic acid, lactic acid prevent or delay the growth of pathogenic bacteria and have been used as preservatives [11].The present work was aimed to preserve cow urine naturally at different pH andto investigate the antibacterial activity of CU at different pH.

Materials And Methods:-

Collection of Cow Urine

Fresh cow urine was collected in a sterile container from Shree Krishan MurariGau-Shala, Dadabari situated at Kota, Rajasthan. The urine was filtered through 5micron filter paper to get rid of debris and precipitate.

Isolation and Identification of Microorganism

The following strains of microbes like *Escherichia coli*, *Staphylococcus aureus*and *Klebsiella sp*. were isolated. Bacteria were identified by colony morphology (shape, structure, color, pattern, size) gram staining, biochemical tests (Indole production, Catalase, Lactose, Methyl red) [12].

Table 1:- Isolation and Identification of Bacterial Strains.

No.	Bacteria	Isolated From	Identification
1.	*E. coli*	Cow Dung	Shape: Rod Gram Staining: -ve Colony: Greenish colonies on EMB Agar Media
2.	*Staphylococcus aureus*	AbscessandotherSkin Lesion	Shape: Cocci Gram Staining: +ve Colony: Golden Yellow colonies on Nutrient Agar media Catalase Test: +ve
3.	*Klebsiella sp.*	Urine	Shape: Rod Gram Staining: -ve Colony: Pinkish Round colonies on MacConkey Agar media Indole Test: -ve

Preparation of inoculums

The bacterial isolates were maintained on nutrient agar and sub-cultured every three days. Each bacterial isolates were suspended in 5 ml of saline solution and adjusted to give a concentration of bacterial cells equivalent to 0.5% McFarland turbidity standard prior to the antibacterial testing [15]

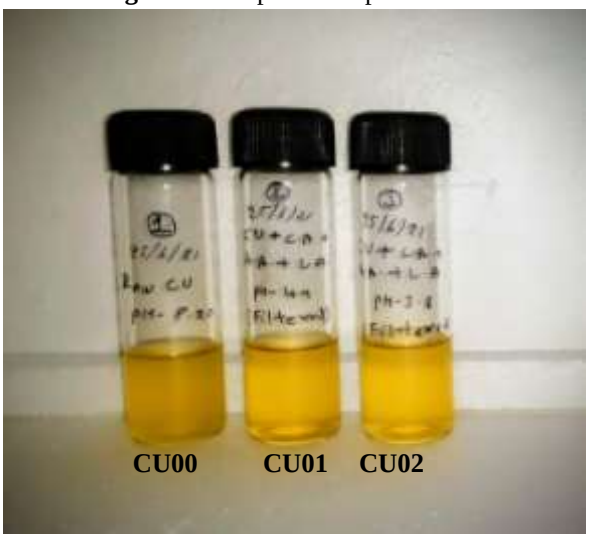
Treatment of CU

Two types of samples were prepared.

1. Sample 1 (CU01)
   To fresh cow urine (pH8.2) samples add Citric Acid to bring pH 6.0. To above add Ascorbic Acid (Vitamin C) to bring pH 5.3 and add Lactic Acid to bring pH 4.4. Filter it through a 5micron filter. Add non caloric sweetener and food color.
2. Sample 2 (CU02)
   To fresh cow urine sample (pH8.2) add 2% Citric Acid to bring pH 4.9. To above add 0.8% Ascorbic Acid (Vitamin C) and add Lactic Acid 1.5% to bring pH.3.8. Filter it through a 5micron filter. Add non caloric sweetener and food color.
3. Sample 3 (CU00)

Filtered fresh cow urine at pH 8.2 (without treatment) was prepared for comparative study.

Figure 1:- Prepared samples of CU



CU00: Fresh CU (pH 8.2)
CU01: Treated and filtered (pH4.4)
CU02: Treated and filtered (pH 3.8)
Lactic acid was added to treated CU for lowering the pH which also eliminates ammonia and urea, making the treated CU odourless and palatable.

To check microbial growth in the treated CU samples.
The sterile plates were prepared and spreaded with 0.5ml of cow urine samples and incubated at 37˚C for 48 hours with a negative control (autoclaved distilled water). This process was repeated every 10 days to check microbial growth in CU.

Antibacterial screening of the treated cow urine samples
Antimicrobial activity of CU was evaluated by the Agar well diffusion method according to the National Committee for Clinical Laboratory Standards (NCCLS) [13]. The inhibition zones were reported in millimeters (mm) and were used as references for the antimicrobial assay of CU.

Briefly, Nutrient agar plates were inoculated with bacterial strain under aseptic conditions. Subsequently, wells of 8mm diameter were punched with a sterile cork borer, and a volume of 35 μL of the CU is introduced into the well and allowed to diffuse at room temperature for 2 h.

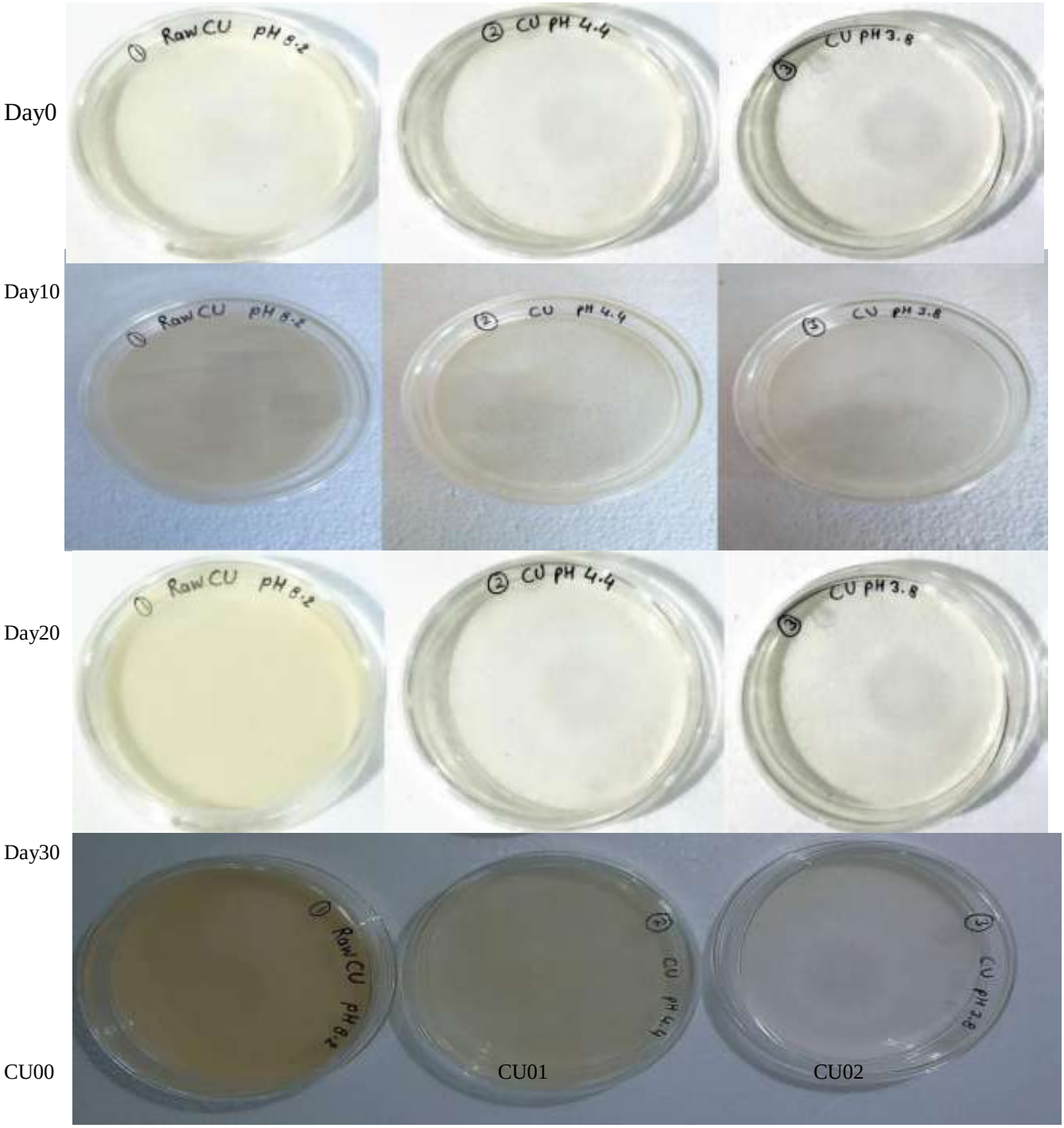
The plates were then incubated at 37℃ for 24 h. For comparison raw cow urine was also used. Sterile distilled water was used as negative control. Antibiotics, Streptomycin was used as a positive control. The antibacterial activity was assessed in agar plates based on the size of the inhibition zone diameter surrounding the wells. For each test, three replicates were performed.

Results and Discussion:-

The inhibitory effects of fresh cow urine at different pH (8.2,4.4,3.8) were analyzed at the intervals of 5,10, 20,30 days (Table 1, Fig.2). Fresh cow urine sample having pH 8.2 (CU00) showed maximum bacterial growth compared to other two samples.

This shows that shelf life of untreated fresh cow urine is short. Treated cow urine samples at low pH showed minimum growth (CU02 > CU01).At pH 3.8 no bacterial growth was found after 30 days.

Figure 2:-Bacterial growth in Cow urine samples



CU00: fresh cow urine at pH 8.2,**CU01**: fresh cow urine at pH 4.4. **CU02:** fresh cow urine at pH 3.8

Cow urine, photoactivated and distillates exhibited antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Proteus vulgaris* (7,18). Antibacterial activity was analyzed against the *Escherichia coli*, *Staphylococcus aureus* and *Klebsiella sp* (Table 2, Fig.3,Fig.4). Fresh cow urine samples at pH 8.2,4.4,3.8 were taken.Maximum antibacterial activity was shown in fresh CU sample (CU02) at pH 3.8 in *Escherichia coli* (24mm ± 2.0), *Staphylococcus aureus* (22mm ± 2.0) and

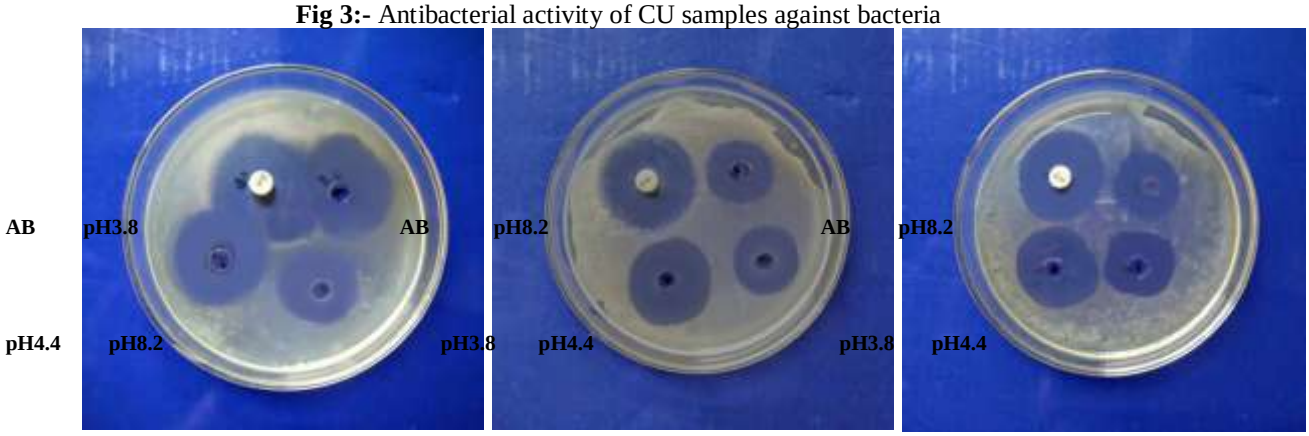
Klebsiella sp (22mm ± 1.0). Cow urine samples (CU01) at pH 4.4 and were also found to show considerable zone of inhibition and CU00 (pH 8.2) also showed considerable zone of inhibition to some extent (for a short time).) Phenols are present in cow urine which are bactericidal to gram positive and gram-negative bacteria (15). This may be the reason for antibacterial activity. Cow urine distillate was also proved to be a bioenhancer for antimicrobial activity (16,17).

Table 2:- Microbial growth in CU at different pH at regular intervals.

No.	Sample Code	Day 0	Day 10	Day 20	Day 30
1.	CU00	-	++	+++	++++
2.	CU01	-	+	+	++
3.	CU02	-	-	-	-

CU00: fresh cow urine at pH 8.2,**CU01**: fresh cow urine at pH 4.4. **CU02:** fresh cow urine at pH 3.8.
'+' shows positive growth of microbes in CU
'-' shows no growth of microbes in CU

Fig 3:- Antibacterial activity of CU samples against bacteria



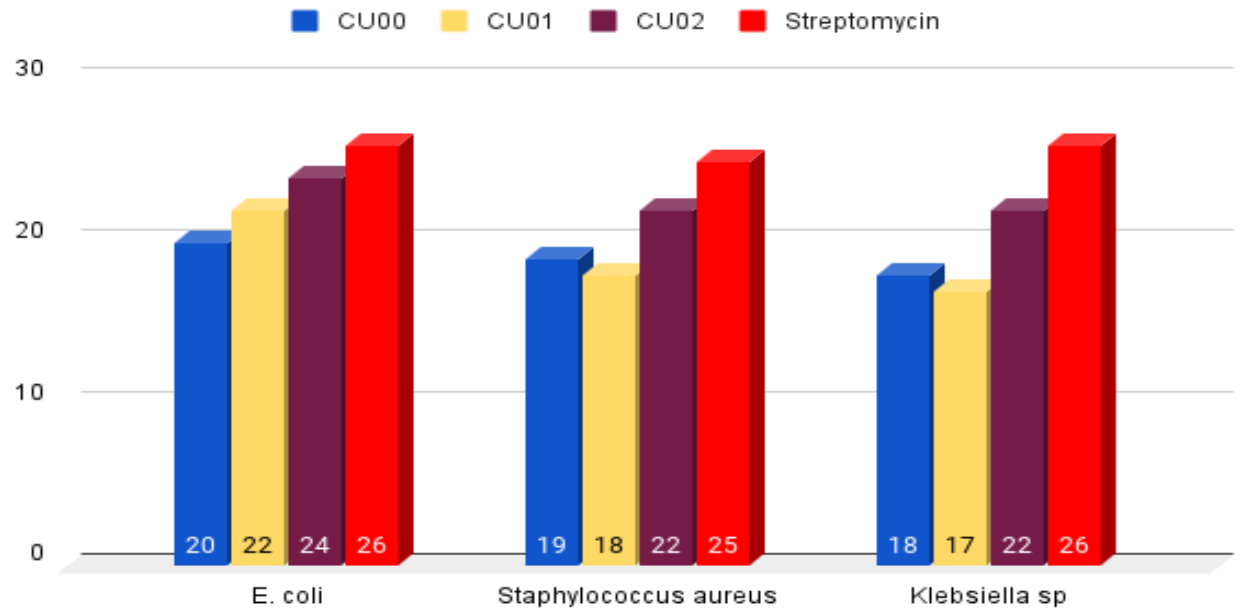
E.coli ***S.aureus*** ***Klebsiella sp***

CU00: fresh cow urine at pH 8.2,**CU01**: fresh cow urine at pH 4.4. **CU02:** fresh cow urine at pH

Table 3:- Antibacterial activity of the treated fresh cow urine samples.

		E.coli	*Staphylococcus aureus*	*Klebsiella sp.*
CU Samples	CU00 Fresh Raw Cow Urine pH 8.2	20mm ± 1.0	19mm ± 1.0	18mm ± 1.0
	CU01 pH 4.4	22mm ± 1.0	18mm ± 1.0	17mm ± 2.0
	CU02 pH 3.8	24mm ± 2.0	22mm ± 2.0	22mm ± 1.0
Antibiotic	Streptomycin	26mm	25mm	26mm

Fig.4:- Antibacterial activity of the treated fresh cow urine samples



Conclusion:-

Gomutra therapy provides an especially rich and provocative research topic. It is effective, eco-friendly with no toxic effects, economical and easily available treatment bestowed upon us by nature. Pathogenic bacteria are resistant to at least one of the drugs for the treatment, care is to be taken from traditional/indigenous medicine to tackle it urgently. Asian communities are using spices, cow urine and medicinal plant extracts in their diets and naturopathy. The Indian traditional knowledge emanates from Ayurveda, where *Bos indicus* is placed at a high pedestal for numerous uses of its various products. Urine is one of the products of a cow with many benefits and without toxicity.

During an investigation on the preservation of cow urine, an Ayurvedic medicine,it was found that it has excellent activities against both gram-positive and gram-negative bacteria at low pH i.e 3.8. The germ -killing activity could be attributed to the presence of some low molecular weight phenolic compounds in it.Lowering pH reduces ammonia and urea content of cow urine and makes it odourless and palatable.

However, there is still a need to explore further research in agrarian countries like India, where the majority of the rural population have cows as their additional source of income. Let's hope this urine therapy could open doors for curing a wide range of dreadful diseases because as we know it is eco-friendly, economically viable, and easily available in abundance.

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Conflict

The authors declare that they have no conflicts of interest.

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