



## ESTIMATION OF CALCIUM LEVELS AND ANTIMICROBIAL ACTIVITY OF COW URINE –AN INVITRO STUDY

### Microbiology

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### ABSTRACT

**INTRODUCTION:-** Cow urine is found to have various therapeutic values. This short study was done to find the amount of Calcium in Cow urine and its Antimicrobial activity on strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis* and *Candida albicans*.

**MATERIALS AND METHOD:-**Total of 30 cow urine samples were collected in sterile wide mouth containers from 5 villages (Nuglipalya, Gidadakone halli, Ullal-Bangalore rural, Benchkalpalya, and Mudhenpalya). The Cow Urine samples were sent to Robust Materials Technology Private Limited for Analysis of Calcium by Atomic Absorption Spectrometry. Antimicrobial susceptibility testing was done by Well-Diffusion method.

**RESULT:-** Estimation of calcium levels showed an average concentration of 363.575 mg/L. 63.3% of the total number of cow urine samples tested showed antimicrobial activity against *Candida albicans* and 13.3% of the sample tested showed antimicrobial activity against *Staphylococcus aureus*.

**CONCLUSION:-**Calcium being a potential central regulator in wound healing in skin and its significant presence in cow urine, in addition to its Antimicrobial activity shows that Cow Urine has potency to be considered for Topical Application in case of Skin Wounds. Further Invivo aswell as Invitro studies need to be conducted to attribute its importance in therapy of complicated wounds.

### KEYWORDS

Cow urine, Antimicrobial activity, Calcium level, Wound healing

### INTRODUCTION:

In Veda, cow is considered the most valuable animal and is called Mother of all. Different products obtained from cow such as urine, dung, milk, ghee and curd are used widely in number of Ayurvedic formulation<sup>(1)</sup>The cow urine is useful in number of disease particularly in gulma, filaria, cancer etc. It is also used with herbs to cure diseases like fever, epilepsy, anemia, abdominal pain, constipation, etc. by the traditional healers. Immunomodulatory, hypoglycemic and cardio-respiratory effects<sup>(2)</sup>. Cow urine is found to have various therapeutic values. It is also known to show antibacterial activity, antifungal activity and shows wound healing properties etc.<sup>(3)</sup>. According to the review done by Randhawa et al, Presence of urea, creatinine, swam kshar (aurum hydroxide), carboic acid, phenols, calcium, and manganese has strongly explained the antimicrobial and germicidal properties of Cow Urine.<sup>(4)</sup>The objectives of our study was to find out Antimicrobial activity of cow urine and also the Calcium level in urine.

### MATERIALS AND METHODS:

This was an in-vitro study which was carried out during the period of June 2017 to December 2017. Antimicrobial activity of Cow urine was tested against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*. The Cow Urine samples were also sent to Robust Materials Technology Private Limited for Analysis of Calcium by Atomic Absorption Spectrometry.

### COLLECTION OF COW URINE SAMPLE:

Total of 30 cow urine samples were collected in sterile wide mouth containers from 5 villages (Nuglipalya, Gidadakone halli, Ullal-Bangalore rural, Benchkalpalya, and Mudhenpalya). Random sampling method was chosen for collection of samples.<sup>(2)</sup>

### ESTIMATION OF CALCIUM CONTENT IN COW URINE SAMPLES:

The samples were sent to Robust materials and technology Pvt.Ltd. Atomic absorption spectrophotometry was the method used.

### MICROBIAL CULTURE:

Strain of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus*

### Antimicrobial Activity Cow Urine Against Tested Microorganism

MICROORGANISM/COW URINE	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16
E.coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
E.faecalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
P.aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

*aureus* and *Pseudomonas aeruginosa* were procured from KLE Society's Dental hospital and maintained on Nutrient agar and *Candida albicans* on Sabouroud's Dextrose agar.

### PREPARATION OF INOCULUM:

The assay was performed according to the standards of the Clinical and Laboratory Standards Institute [CLSI]. The isolates of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* was taken and inoculated in peptone water incubated for 4 hours at 37°C. Inoculum was adjusted to 0.5 Mc Farland standards (1.5x10<sup>8</sup> cfu/ml).

### ANTIMICROBIAL ACTIVITY SCREENING BY AGAR-WELL DIFFUSION METHOD:

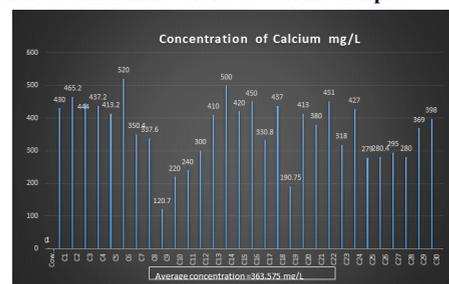
The inoculum was swabbed uniformly on to solidified Mueller-Hinton Agar and was allowed to dry for 5 min. Wells of 6 mm in diameter were made in the seeded agar using sterile cork borer. 100 µl of the cow urine sample was inoculated in each well in sterile condition. The plates were then incubated at 37°C for 24hrs for bacterial plates and 48hrs for fungal plates. The zone of inhibition was measured in millimeter.<sup>(5)</sup>

### pH ANALYSIS OF COW URINE:

The samples were sent to Robust materials and technology Pvt.Ltd. IS 3025(Part-11):1983 method was used for pH analysis.

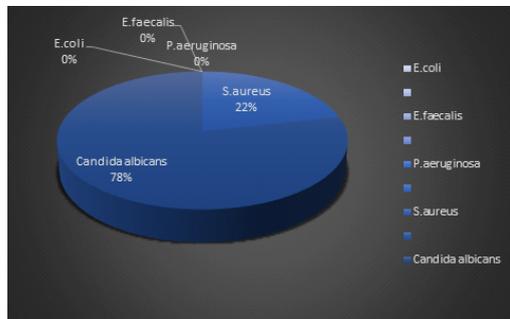
### RESULTS:

#### Estimation Of Calcium Levels In Cow Urine Samples:-



S.aureus	19	22	R	R	R	R	21	R	R	R	R	R	R	R	R
C.albicans	13	16	R	14	16	16	20	R	R	R	13	12	R	R	R
MICROORGANISM/COW URINE	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	
E.coli	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
E.faecalis	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
P.aeruginosa	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
S.aureus	R	R	R	R	R	R	R	R	R	20	R	R	R	R	
C.albicans	17	16	20	20	R	R	14	16	18	14	R	R	14	14	

ZONE OF INHIBITION (in mm)  
C1-C30=COW URINE SAMPLES



**AVERAGE pH VALUE:** - Average pH value was found to be 7.76.

**DISCUSSION:**

19 Cow urine samples out of 30 (63.3%) showed antimicrobial activity against *Candida albicans*. 4 out of 30 cow urine samples (13.3%) showed antimicrobial activity against *Staphylococcus aureus*. In our study it shows that cow urine had more action on *Candida* compared to other organism. It also proven in a study that 75% of the clinical isolates tested were sensitive to Cow urine. MIC of Cow urine against *candida albicans* is as low as 25%<sup>(6)</sup> Considering estimation of concentration of calcium in Cow urine this study shows considerably high average concentration of calcium which is 363.575 mg/L when compared to the study by Parihar et al., who had obtained 82.02 mg/L of calcium by atomic absorption Spectrometry and a review of cow urine by Gulhane Harshad et al also mentions calcium level in as 0.1-1.4ml/kg/day<sup>(7)(8)</sup>. Wound healing is a calcium-mediated process. Clinically, the direct topical application of calcium to chronic human wounds through calcium alginate dressings has shown to be beneficial<sup>(9)</sup>. In vivo studies have also been done against wound healing property of cow urine on wistar albino rats which has shown positive results<sup>(10)</sup>. Current study shows that cow urine has considerable amount of calcium. *Staphylococcus aureus* and *Candida albicans* are one of the few important bacteria and fungus respectively isolated from sites of wound<sup>(11)(12)(13)</sup>. Hence cow urine can be considered for topical applications in case of wounds and healing procedure could be related to considerably good amount of calcium present in cow urine.

**CONCLUSION:**

Calcium being a potential central regulator in wound healing in skin and its significant presence in cow urine, in addition to its Antimicrobial activity shows that Cow Urine has potency to be considered for Topical Application in case of Skin Wounds. Further In vivo as well as Invitro studies need to be conducted to attribute its importance in therapy of complicated wounds.

**REFERENCES**

- Charmi P Shah, Deval M Patel, Paras D Dhami, Janak Kakadia, Dhruvesh Bhavsar, Urmila D Vachhani, Manisha N Trivedi, Joshi V J, In Vitro Screening Of Antibacterial Activity Of Cow Urine Against Pathogenic Human Bacterial Strains, International Journal Of Current Pharmaceutical Research, Vol3, Issue 2, 2011.
- S. Raad , D.V. Deshmukh, S. N. Harke and M.S. Kachole , Antibacterial Activity Of Cow Urine Against Some Pathogenic And Nonpathogenic Bacteria, Raad Et Al., Ijpsr, 2013; Vol. 4(4): 1534-1539.
- Sonia Singla and Satwinder Kaur. Biological Activities of Cow Urine: An Ayurvedic Elixir. Ejjpmr, 2016, 3(4), 118-124.
- Gurpreet Kaur Randhawa and Rajiv Sharma Chemotherapeutic Potential of Cow Urine: A Review J Interclut Ethnopharmacol. 2015 Apr-Jun; 4(2): 180–186.
- Maher Obeidat, Mohamad Shatnawi, Mohammad Al-Alawi, Enas Al-Zu'Bi, Hane Al-Dmoor, Maisa Al-Qudah, Jafar El-Qudah and Ismael Otri, Research Journal Of Microbiology, Volume 7 (1): 59-67, 2012
- Jian Meng Hoh, B. Dhanashree. Antifungal Effect of Cow's Urine Distillate on *Candida* Species. Journal of Ayurveda and Integrative Medicine Xxx (2017) 1e5
- Http://Shodhganga.Inflibnet.Ac.In/Bitstream/10603/6929/6/06\_Chapter%202.Pdf
- Gulhane Harshad , Nakaneekar Amit , Mahakal Nilesh , Bhople Sunanda , Salunke Amrnat , Gomutra (Cow Urine): A Multidimensional Drug Review Article, Gulhane Harshad Et Al/Int. J. Res. Ayurveda Pharm. 8 (5), 2017
- Kenichiro Kawai, Barrett J. Larson, Hisako Ishise, Antoine Lyonel Carre, Soh Nishimoto, Michael Longaker, And H. Peter Lorenz., Calcium-Based Nanoparticles

- Accelerate Skin Wound Healing Plos One. 2011; 6(11): E27106.
- Sanganal Js Et Al., Effect Of Cow Urine On Wound Healing Property In Wistar Albino Rats. Veterinary World 2011; 4(7): 317-21
- Kemebadikumo Pondei, Beledanyo G. Fente and Oluwatoyosi Oladapo, Current Microbial Isolates From Wound Swabs, Their Culture And Sensitivity Pattern At The Niger Delta University Teaching Hospital, Okolobiri, Nigeria, Tropical Medicine And Health Vol. 41 No. 2, 2013, Pp. 49-53
- A. Raz-Pasteur, Y. Ullmann and I. Berdicevsky. The Pathogenesis of *Candida* Infections in a Human Skin Model: Scanning Electron Microscope Observations, International Scholarly Research Network Ism Dermatology Volume 2011, Article Id 150642, 6 Pages
- Gilmara Celli Maia Almeida, Marquiony Marques Dos Santos, Nara Grazieli Martins Lima, Thiago André Cidral, Maria Celeste Nunes Melo and Kenio Costa Lima, Prevalence And Factors Associated With Wound Colonization By *Staphylococcus* Spp. And *Staphylococcus Aureus* in Hospitalized Patients in Inland Northeastern Brazil: A Cross-Sectional Study, Almeida Et Al. BMC Infectious Diseases 2014, 14:328