



## ANTIFUNGAL ACTIVITY OF COW URINE AGAINST ENVIRONMENTAL ISOLATES

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### Abstract

Increase in resistance among fungal species, to routinely used antifungal agents has necessitated the quest for new drugs. Few studies have revealed that cow's urine can suppress the growth of pathogenic fungi. However, there is limited published report on antifungal effects of formulated cow's urine on fungal isolates. The present study aims at exploring the antifungal potential of cow's urine on various fungal isolates. In this *in-vitro* experimental study five fungal genera were tested for their susceptibility to amphotericin B, fluconazole and voriconazole and cow urine by well diffusion and point inoculation method. Detection of inhibitory efficacy of cow's urine for the fungal isolates was done by agar dilution method using 5–20% concentration of cow's urine. The environmental isolates of 5 genera: *Aspergillus niger*, *Candida albicans*, *Penicillium spp.*, *Fusarium spp.* and *Rhizopus spp.* were tested against cow urine for their antifungal susceptibility. The results revealed that the effectiveness was found highest against the fungal spp. of *Candida spp.* (90%) and *Penicillium spp.* (78%) followed by *Aspergillus niger* (70%), *Fusarium spp.* (65%) and *Rhizobium spp.* (60%). It was found that the isolates were less sensitive to Itraconazole and fluconazole while found highly susceptible to cow urine treatment. The study concludes that Cow's urine distillate has effective inhibition on fungal species and with a few modifications this can be a good natural alternative for reducing several diseases.

**Key words:** *Antifungal agents, Fungal species, Cow's urine, effectiveness, natural product*

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## Introduction:

From the ancient times and till to the present, cow urine has been used as a medicine in the traditional Indian system. In Veda's urine of cow is compared to the nectar and said to be most effective secretions of animal origin with innumerable therapeutic values (Cow urine manual) The studies had shown that older the cow urine, the more is its benefits. In Indian traditional system Gomaata (Female cow) is considered as a sacred animal. From ancient era Gomutra (cow urine) has been used to treat several diseases of human and the Indian Ayurveda literature 'Charaksamhita' and 'Shushrutsamhita' described the different rare medicinal properties of Gomutra. In modern research much experimental evidence supports to antimicrobial, antidiabetic, anticancer activity of Gomutra [5-7]. Cow urine does not decay as it contains copper and gold salt which are elixirs, and this is already known that Gold has capability of curing many diseases. Cow urine destroys miseries and disease, simultaneously helps in the purification of both mind and body (R. kavya). A survey carried out by Kiwuso *et. al.*, in 2004 stated that the cow urine, wood ash and red pepper have important role in controlling termites [1]. Nargis Akhtar (2006) studied the inhibitory effect of some plant extract and cow urine plant pathogen [2]. The antibacterial properties were also reported from *Dalbergia sisso* & *Datura stramonium* in combination with cow urine against pathogenic gram+ & gram-bacteria. [3]. Thus, the present study was designed and undertaken to investigate the antifungal activity of cow urine. Although a lot of research and development is going on for improving the treatment strategies for curing fungal diseases, but a limited number of drugs are available in the market to win this battle against fungal infections. Use of antifungal to cure fungal infections is a common worldwide accepted medical practice. But from last few decades, management of infectious fungal infection has become difficult due to the

development of resistance and limited numbers of antifungal compounds [4]. The present study focused on antifungal potential of treated cow urine against common fungal pathogens.

## Material And Method:

### Collection of CU (Cow Urine)

Fresh cow urine was collected in a sterile container from Gau gram organic products situated at Kota, Rajasthan. The urine was filtered through 5micron filter paper to get rid of debris and precipitated material.

### Treatment of CU

To fresh Cow urine sample (pH 8.2), 2% Citric Acid is added to bring pH from 8.2 to 4.9. To the above prepared sample 0.8% Ascorbic Acid (Vitamin C) and 1.5% Lactic Acid is added to make it of pH.3.8. Now this prepared sample is filtered through a 5micron filter. Non caloric sweetener and food color is added afterwards [7].

### Preparation of Sabouraud glucose agar medium with chloramphenicol

– Casein enzyme hydrolysate - 5 gm / lit. Peptic digest of animal tissue - 5 gm / lit. Dextrose - 40 gm / lit. Chloramphenicol - 0.05 gm/lit Agar - 15 gm / lit.

### Test Fungi

The test fungi were as follows – *Fusarium spp.*, *Rhizopus spp.*, *Aspergillus niger*, *Penicillium notatum* and *Candida albicans*.  
**Antifungal activity of cow urine [7]**

The pure culture of fungi was isolated environmentally and maintained on Sabouraud chloramphenicol agar medium. Culture was maintained in refrigerator for use and regularly checked for contamination. Periodic transfers were made aseptically.

### Spore suspension Preparation

A small aliquot of distilled water was taken in a clean test tube and plugged properly.

All the materials were sterilized at 121<sup>0</sup> C for 15 mins. at 15 lbs pressure. After sterilization, the tubes were cooled down and a few loopful sporulated fungal pure culture were transferred to the tubes containing sterilized distilled water. After proper mixing, the suspension was used for antifungal assay using point inoculation method.

### Screening for antifungal activity [7]

#### Point inoculation method

SDA Medium was prepared, Poisoned with varying concentrations of urine samples. All the experiments were performed in triplicates. Positive and negative controls were also included in the studies. After solidification of the medium, the test fungi were inoculated by point inoculation method under aseptic conditions without dispersing the inoculum. The plates were then incubated in upright position at room temperature (25 to 27<sup>0</sup>C) for 48-72hrs. The diameter was measured and enlisted in table 1.

#### Well Diffusion Assay

The antifungal assay was determined by growing the fungal spores in the SD broth. 48hrs. old culture was spread over the SDA plates. 6mm well were punched in the agar with the help of cork borer. 100 microliters of the different cow urine samples were used in the wells and incubated for 48hrs. The positive control was used an antifungal drug; fluconazole and the solvent used was considered as negative control. The Zone diameter of inhibition were recorded and enlisted in Table-2. The zones recorded were calculated using mean $\pm$  SD as the experiments were performed in triplicates [7].

### Result and Discussion

All the isolates were sub cultured and grown in broth culture or spore inoculation was performed. Antifungal activity was processed in terms of two different

methods. After 3 days incubation the plates were observed, and the evaluation of antifungal efficacy was reported by the effect of cow urine. The results revealed that the cow urine showed clear zone of inhibition against all tested fungal cultures (Table 1 and table 2). The data obtained was enlisted as qualitative investigation likely to indicate the presence of potent antifungal molecule that is present in cow urine. A lot of work on the beneficial applications of cow urine is under study and many researchers had investigated antifungal potential of cow urine against same fungal pathogens, but as compared to the results of other researchers the results obtained in the present investigation showed more potential of cow urine. From the data of both tables, it was very clear that the Cow urine has high efficacy against *Candida spp.* and *Penicillium spp.* followed by *A. niger*. Shaha *et. al.*, analyzed the potency of cow urine against bacterial pathogens they found that fresh cow urine is more potent than stored cow urine because during storage volatile bioactive compounds may lose [8]. Rakesh, *et. al.*, analyzed the cow urine against agricultural important fungal pathogens, the value of antifungal zone diameter against *Fusarium sp.* is slightly higher than present investigation [9-10] is because of difference between two tested strains but the results obtained from present study against *Fusarium spp.* are enough to support efficacy of cow urine against clinical as well as agricultural invasive fungal infections [11].

### Conclusion

Cow urine is a natural factory of variety of diverse group of bioactive compounds but when this comes to comparison to plant derived product the research on cow urine is less. The researchers who have done research on cow urine from last decades makes a way to support that cow urine have ability to successfully fight many dangerous infections. On other hand as a natural product of Cow the use of cow urine

**Table- 1: Antifungal activity of different concentrations of cow urine**

S. NO.	Test fungus	Negative Control (C.D.)	5% ICU (C.D.)	10% ICU (C.D.)	15% ICU (C.D.)	20% ICU (C.D.)	Positive Control (C.D.)
1.	<i>Fusarium spp.</i>	4.8 cm	3.2 cm	3.1 cm	2.8 cm	1.8 cm	1.4 cm
2.	<i>Rhizopus spp.</i>	5.9 cm	3.9 cm	3.8 cm	3.4 cm	2.2 cm	1.9 cm
3.	<i>Aspergillus niger</i>	3.8 cm	2.5 cm	2.4 cm	2.3 cm	1.8 cm	0.9 cm
4.	<i>Penicillium notatum</i>	3.2 cm	2.4 cm	1.7 cm	1.0 cm	No Growth	1.2 cm
5.	<i>Candida albicans</i>	2.6 cm	1.2 cm	0.8 cm	0.4 cm	No Growth	0.6 cm

**Table- 2: Antifungal activity of different concentrations of cow urine (Well diffusion Assay)**

S. NO.	Test fungus	Negative Control (ZDI)	5% CU (ZDI)	10% CU (ZDI)	15% CU (ZDI)	20% CU (ZDI)	Positive Control (ZDI)
1.	<i>Fusarium spp.</i>	NZ	10±2	11±1	15±2	21±1	18±2
2.	<i>Rhizopus spp.</i>	NZ	9±2	10±1	12±2	15±0.3	16±1
3.	<i>Aspergillus niger</i>	NZ	11±2	12±1	14±2	16±1	21±2
4.	<i>Penicillium notatum</i>	NZ	14±1	17±1	15±2	19±2	18±2
5.	<i>Candida albicans</i>	NZ	16±1	18±1	20±2	22±1	23±2

**Fig 1. Media plates showing antifungal effect of cow urine**

urine is cost effective as well as environment friendly and safe for mankind. The present qualitative analysis concluded that cow urine can be used to control or minimize the fungal infections and much more study is required on higher levels to know the actual lead compounds that is present in cow urine and act against fungal pathogens.

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