

## Antimicrobial Activities of Cow Urine Distillate Against Some Clinical Pathogens

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**Abstract:** From the ancient period cow's urine has been used as a medicine. In India, drinking of cow urine has been practiced for thousands of years. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely Urine, Dung, Milk, Ghee and Curd. The present study analyzes the antibacterial and antifungal activity of Cow Urine Distillate against the clinical pathogenic microorganisms. Antibacterial activity of Cow Urine Distillate (5, 10 and 15 $\mu$ l) was analyzed against the *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi*. Maximum antibacterial activity was observed in *Pseudomonas aeruginosa* (7.06 $\pm$ 0.05, 8.08 $\pm$ 0.18 and 10.4 $\pm$ 1.23, mm in diameter, respectively) and *Salmonella typhi* (6.3 $\pm$ 1.23, 8.06 $\pm$ 0.17 and 10.4 $\pm$ 1.2, mm in diameter, respectively). Antifungal activity of cow urine distillate was analysed against *Aspergillus niger* and *Aspergillus flavus*. When the two fungal organisms were compared, maximum growth suppression was observed in *Aspergillus niger* (3 $\pm$ 0.14, 6.3 $\pm$ 1.2 and 7.06 $\pm$ 0.04, mm in diameter) than *Aspergillus flavus* (2.03 $\pm$ 0.25, 4.9 $\pm$ 0.26 and 6.3 $\pm$ 1.2, mm in diameter, respectively). Finally concluded that the cow urine distillate has antibacterial and antifungal activities and the inhibitory activity can be used in the control of bacteria and fungi of various origins.

**Key words:** Cow Urine Distillate • Antibacterial and Antifungal Activity

### INTRODUCTION

In Veda, cow's urine was compared to the nectar. In substrata, several medicinal properties of cow's urine have been mentioned and are known to cause weight loss, reversal of certain cardiac and kidney problems, indigestion, stomach ache, edema, etc. Cow urine has a unique place in Ayurveda and has been described in 'Sushrita Sumhita' and Ashtanga Sangraha' to be the most effective substance secretion of animal origin with innumerable therapeutic values. It has been recognized as water of life or "Amrita" (Beverages of immortality), the nectar of the God. In India, drinking of cow urine has been practiced for thousands of years. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely Urine, Dung, Milk, Ghee and Curd. A number of formulations mentioned in Ayurveda describe the use of Panchagavya components either alone or in combination with drugs of herbal, animal or mineral origin [1].

An exhaustive reference of cow's urine having curative properties in skin diseases, especially leprosy, is referred to in *Caraka samhita*. Furthermore, in the

treatment of falling body parts, discharging lymphs and organism infested organs, use of cow's urine (along with some other ingredients) has been recommended for bath, anointing and intake [2]. The cow urine distillate has been patented as activity enhancer and availability facilitator for bioactive molecules including anti-infective and anti-cancer agents (US Patent No 6410 059/2002) [3]. Chakra pani mishra in vishva vallabba recommends using extracts of cow urine in herbal insecticides [4]. Feeding of cow urine increased the feed intake in white legborn layers [5]. The present study was carried out to prepare cow urine distillate and to determine the antibacterial and antifungal activities.

### MATERIALS AND METHODS

**Collection of Sample:** Cow urine sample was collected from cow farm (at Avanam, Thanjavur Dt) using sterile container and stored for further uses.

**CUD Preparation:** Cow urine was distilled at 100°C using distillation apparatus [6]. The single distilled cow urine was acidified by lowering the pH below 2.0 with the

addition of 85% orthophosphoric acid. The cow urine was again distilled at 100°C using a distillation apparatus to remove ammonia. The distillate was stored in sterile glass flask at refrigerator (4°C).

**Test Organisms:** Bacterial and fungal cultures were used as test organisms *Bacillus subtilis* (MTCC 7415) *Pseudomonas aeruginosa* (MTCC 7436), *Klebsiella pneumoniae* (MTCC 7407), *Salmonella typhi*, *Aspergillus niger* and *Aspergillus flavus* were collected from microbial type culture collection centre (MTCC) at Chandigarh.

**Disc Preparation:** 5 mm (diameter) discs were prepared from whattman No.1 filter paper. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture discs were dried on hot air oven at 50°C. The sterile discs were rinsed in cow urine distillate at different concentration (5, 10, 15µl).

**Antibacterial and Antifungal Activity of Cow Urine Distillate:** The antimicrobial and antifungal activity studies were carried out by disc diffusion technique [7]. The sterile Mueller Hinton agar plates were prepared. The test organisms like *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Aspergillus niger* and *Aspergillus flavus* were spreaded over the Mueller Hinton agar plates by using separate sterile cotton swabs. After the spreading the different concentrated cow urine distillate discs were placed separately on the organism inoculated

plates with equal distance positive control disc was also maintain. All the bacterial plates were incubated at 37°C for 24hrs and fungal plates at 24°C for 72 hrs. After incubation the diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

**Statistical Analysis:** Mean and standard deviation were calculated to facilitate the comparison of the data [8].

## RESULTS AND DISCUSSION

**Antibacterial Activity:** Antibacterial activity of cow urine distillate was analyzed against the *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Salmonella typhi* (Table 1, Fig. 1 and Plate 1). 5, 10 and 15µl concentrations of cow urine distillate discs were taken for the study. Among the three concentrations highest antibacterial activity was noted in 15µl concentration when compared with 5 and 10µl. Maximum antibacterial activity was observed in *Pseudomonas aeruginosa* (12.6±0.05, 13.8±0.18 and 15.4±1.23, mm in diameter, respectively) and *Salmonella typhi* (12.3±1.23, 13.6±0.17 and 15.4±1.23, mm in diameter, respectively) when compared with other bacterial species and the standard antibiotic (Ampicillin). US patent was obtained by CSIR (Council for Scientific Industrial Research) India which claimed a novel pharmaceutical composition present in cow urine distillate and is effective as an antifungal and antibacterial [6].

Table 1: Antibacterial activity of cow urine distillate

		Zone of inhibition (mm in diameter) (M±SD) (n=3)			
		Concentration of CUD (µl)			
S.NO.	Bacteria	5	10	15	(Amp*)
1.	<i>Bacillus subtilis</i>	7.6±0.04	8.6±0.17	8.8±0.17	7.1±0.01
2.	<i>Pseudomonas aeruginosa</i>	12.6±0.04	13.6±0.17	15.4±1.23	11.2±0.01
3.	<i>Klebsiella pneumoniae</i>	7.3±0.25	7.3±0.25	11±0.14	9.5±0.05
4.	<i>Salmonella typhi</i>	12±1.23	13.6±0.17	15.4±1.23	9.6±0.02

Values are triplicate mean ± Standard deviation

Amp\* - Standard antibiotic disc Ampicillin (30mg/disc)

Table 2: Antifungal activity of cow urine distillate

		Zone of inhibition (mm in diameter) (M±SD) (n=3)		
		Concentration of CUD (µl)		
S.NO.	Fungi	5	10	15
1.	<i>Aspergillus niger</i>	8±0.14	11.3±1.2	12.6±0.04
2.	<i>Aspergillus flavus</i>	7.3±0.25	10±0.25	11±1.2

Values are triplicate mean ± Standard deviation

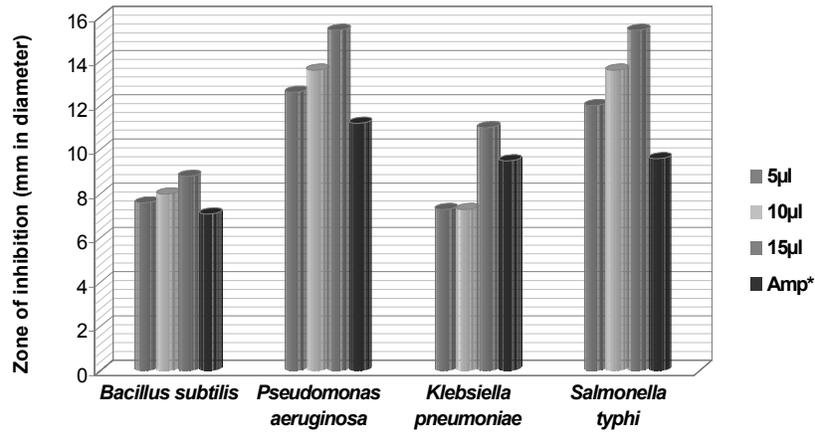


Fig. 1: Antibacterial activity of cow urine distillate

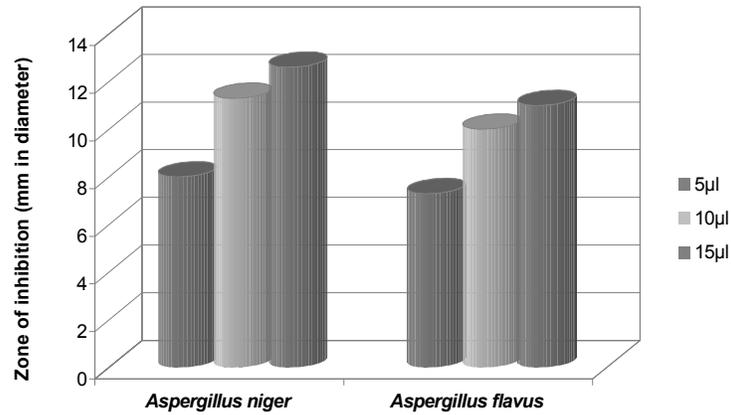


Fig. 2: Antifungal activity of cow urine distillate

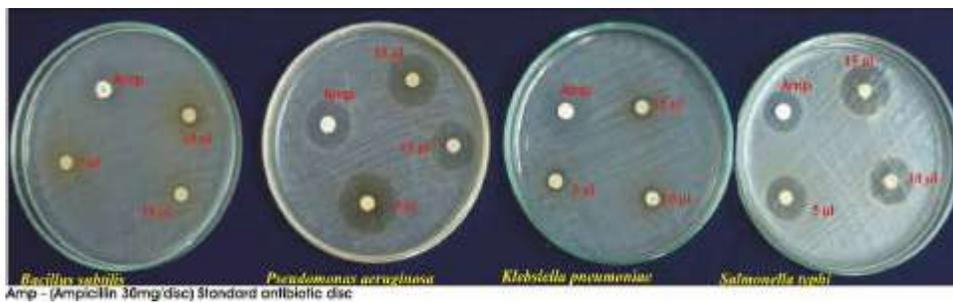


Plate 1: Antibacterial activity of CUD against Pathogenic bacteria



Plate 2= Antifungal activity of CUD against Pathogenic fungi

**Antifungal Activity:** Antifungal activity of cow urine distillate was analysed against *Aspergillus niger* and *Aspergillus flavus*. The investigated results were presented in Table 2, Fig. 2 and Plate 2. When the two fungal organisms were compared, maximum growth suppression was observed in *Aspergillus niger* ( $8\pm 0.14$ ,  $11.3\pm 1.2$  and  $12.6\pm 0.04$ , mm in diameter, respectively) than *Aspergillus flavus* ( $7.3\pm 0.25$ ,  $10\pm 0.26$  and  $11\pm 1.2$ , mm in diameter, respectively). A similar result reported by Prashith Kekuda *et al.* [9] Cow Urine Distillate at various concentrations was tested for antifungal activity. The growth reduction in percentage was taken into consideration and antifungal effect was evaluated. 5% cow urine distillate was more effective against *Mucor sp.* (37.1%) followed by *A.oryzae* (10.2%) and *A. niger* (5.4%).

It was concluded that the cow urine distillate has antibacterial and antifungal activities the inhibitory activity can be used in the control of bacteria and fungi of various origins. The test was done *in vitro*. Same result may be obtained *in vivo* also. Now a day's urinothrapy treatment was developed in the medical sectors. Further studies analyze which components are responsible for antimicrobial activity and animal model could reveal antibacterial and antifungal activity of cow urine distillate *in vivo*.

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