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Research Article

Evaluation of biochemical, antioxidant and antibacterial properties of cow urine

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Abstract

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.

Keywords antibacterial, antioxidant, cow urine, DPPH, paper chromatography

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Introduction

Animal based products have been used in medicines, meta-physical and religious rituals all over the world. Urine of several domestic animals is of therapeutic value in Unani medicine but cow urine is described as the best of all types of animal urine [1]. Medicinal uses of cow urine have been in practice since time immemorial. Cow urine is called as 'Sanjivani' and 'Amrita' in Ayurveda but some people recognized it as "water of life". According to the Chinese pharmaceutical dictionary "Shang Han Lun", cow urine had been used as a medium for delivery of medicinal herbs to strengthen their effects [2].

Cow, *Bos indicus* is one of the most valuable animals in all communities. The cow urine is useful in number of diseases including fever, epilepsy, anemia, abdominal pain, constipation. It has immunomodulatory, hypoglycemic and cardio-respiratory effects. Medicinal usages of cow urine are extensively searched and scientifically endorsed [3].

Cow urine has also been granted US patents (US Patent No. 6896907, 6410059) for its role as an antibiotic, antifungal and anticancer agent [4]. There is an increase in resistance of bacteria against a wide range of antimicrobial agent which is of great concern for the public health professionals. In a case where nearly 70% of pathogenic bacteria are resistant to at least one of the drugs, an urgent attention is required to explore for an alternative. In Indian traditional knowledge, "Gomutra" (cow urine) have found description in several ancient ayurvedic texts like Atharva Veda, Charak Samhita, Sushrut Samhita, Vridhabhagabhatt, Bhavprakash, in maintaining health and combating ailments. Several research findings concluded that cow urine has antioxidant and antibacterial activities [5].

Numerous biomolecules like proteins, peptides and metabolites are present in urine. Urine is formed via glomerular filtration of plasma in the kidneys, which act as a filter to retain most of the plasma proteins [6]. However, many low molecular weight proteins and peptides passing through the glomerular membrane are catabolized in the proximal tubule and then secreted in the urine. In addition, abundant serum proteins such as albumin, immunoglobulin light chain, transferrin, myoglobin, and receptor-associated protein, after passing through the glomeruli, are reabsorbed by endocytic receptors in the proximal renal tubules [7]. Overall, the protein concentration in normal urine is usually very low [8]. Moreover, there is interest in using urine for diagnostic applications due to its simple and non-invasive collection compared to other biological fluids.

Cow urine is considered to be the most effective animal origin substance or secretion having intrinsic property of general health improvement [9]. Free radicals are group of atoms with an unpaired number of electrons and can be formed when oxygen interacts with certain molecules. These are generated by air pollution, smoking and alcohol. The free radicals in our body damage the cell and its cellular components thus it leads to many disorders and cause genetic disease. Antioxidants are the substances that scavenge these free radicals and prevent the damage to the vital molecules [10]. These have the ability to prevent, delay or ameliorate many of the effects of free radicals [11]. The cow urine and its distillate have shown antioxidant activity [12].

Cow urine from a healthy Gir cow, was used to assess the antibacterial effect against *E. coli*, *S. typhimurium*, *P. vulgaris*, *S. aureus* and *B. subtilis* [13]. Similar findings were observed by [12] using Sahiwal cattle. The antibacterial efficacy (as mean zone of inhibition in mm) of cow urine concentrate (CUC) obtained from Karnataka breed, Amritmahal was comparable with Streptomycin on *B. subtilis*, *S. aureus*, *E. coli* and *E. aerogenes* using disc diffusion method [14].

Cow urine is effective in inhibiting the microbial growth and its medicinal values are described in ancient Indian literature. It contains many substances which contribute to its antimicrobial properties. In the present study, physico-biochemical, antioxidant and antibacterial activities of cow urine from indigenous breed were evaluated.

Methodology

Collection of urine sample

Urine samples were collected randomly from six different cows in a sterile wide mouth container from the indigenous breeds (Hariana) reared under standard conditions at Instructional Livestock Farm Complex (ILFC), Mathura and pooled together and stored at 4°C.

Qualitative test of cow urine sample

The qualitative test was performed using the strip test method (Piramal Healthcare ltd.) after centrifugation at 3000 rpm for 10 minutes for the removal of sediments. The supernatant was collected and the parameters like pH, specific gravity, urobilinogen, bilirubin, ketone, blood, protein, nitrite, leukocytes and glucose were observed. Simultaneously, microscopic examination was also done for the presence of casts, crystals, blood, cylindruria.

Lyophilization of the pooled cow urine sample

The pooled cow urine sample was filtered through 0.2mm filter using vacuum filter. The filtered urine sample was then freeze dried using lyophilizer.

Protein estimation

The quantity of protein in fresh cow urine was estimated following the methods of Folin and Lowry [15].

Antioxidant assay (DPPH free radical scavenging activity)

The free radical scavenging capacity of the cow urine was determined using DPPH (2, 2-diphenyl-1-picrylhrazyl) assay [16]. To a set of test tubes, 2.9 ml of DPPH solution (100µg/ml in methanol) and 0.1 ml

of varying concentrations of test samples were added. After mixing the content, it was allowed to dark for 30 minutes and the absorbance was recorded at 517 nm. A control was prepared by using 0.1 ml of methanol and 2.9 ml of DPPH radical solution. Percentage scavenging of DPPH radical was calculated by comparing the absorbance between the sample and control. Ascorbic acid was used as a standard.

% scavenging of DPPH radical = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Paper chromatography

The urine sample was analyzed for the presence of amino acids using paper chromatography technique. A strip of Whatman's filter paper no.1 was cut and approximately 1cm from the end of the length a line was drawn with the help of a pencil. At the center of the line, a tiny spot of the sample was placed. The spot was allowed to dry and then placed in the chamber containing saturated solvent system (Butanol: Acetic Acid: Water, 4:1:5). The chromatogram was allowed to run upto $3/4^{th}$ the paper and then taken out and dried in an oven and then sprayed with locating reagent (Ninhydrin). The R_f value of the spot that appeared was calculated.

Antimicrobial activity

Antimicrobial activities of both fresh and lyophilized cow urine along with oxytetracycline (1mg/ml) were assessed by disc diffusion assay against the Gram negative pathogens.

Test organisms

Clinical isolates of *E. coli* and *Salmonella typhimurium* were used in this study.

Preparation of inoculum

Freshly grown 12 hour old cultures in nutrient broth were used as the inoculums in antibacterial assays.

Disc preparation

Paper disc of filter paper Whatman's No. 1 were prepared. The discs were sterilized by autoclave at 121°C. After the sterilization the moisture on the discs were dried in hot air oven at 50°C. The sterile discs were kept in a pre-sterilized container until further use.

Disc diffusion assay

Disc diffusion assay was used to analyze the antimicrobial activity of the urine samples against the clinical isolates. In a petri plate 15 ml of nutrient agar was poured and allowed to solidify. 100 µl of 12-hour old bacterial culture was inoculated on the solidified agar. Pre-sterilized Whatman no. 1 paper discs (6mm) saturated with urine were placed on it. The plates were kept at 4°C for 10 minutes and then incubated at 37°C for 24 hours. The antibacterial activity was measured using the zone of inhibition around the discs.

Results and Discussion

Qualitative test of cow urine sample

The parameters like pH, specific gravity, urobilinogen, bilirubin, ketone, blood, protein, nitrite, leukocytes and glucose were observed as normal. The microscopic examination revealed normal urine.

Protein Quantification

The amount of protein estimated in the fresh cow urine samples was found to be 0.66±0.11 g/dl.

Antioxidant activity

The reduction capability of DPPH radicals was assessed by the reduction in its absorbance at 517 nm. The DPPH radical scavenging activity of the fresh cow urine increases with increasing concentration (Table 1).

Table 1. DPPH free radical scavenging activity

Sl. No.	Samples (µl/ml)	% Scavenging of DPPH at 517nm					
		100	200	400	600	800	1000
1.	Fresh cow urine	19.1±0.14*	30.7±0.27	48.2±0.20	56.1±0.14	60.8±0.20	70.6±0.32

*Values are mean±SE of three replicates.

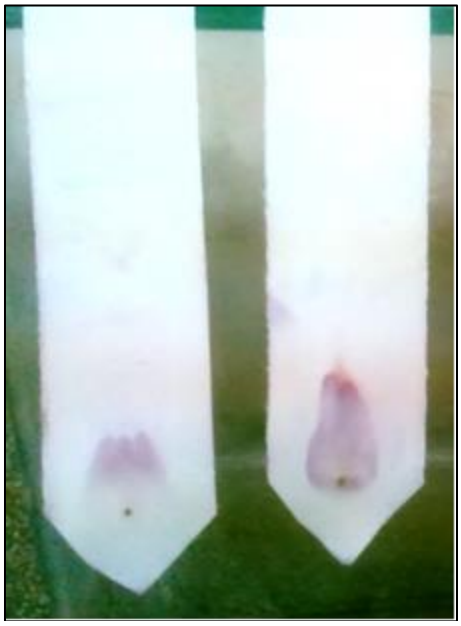

Figure 1. Paper chromatography of fresh cow urine

Paper Chromatography

The chromatogram was observed for the presence of spot and the Rf value of the spots were analyzed (Figure 1). The calculated R_f value indicated the presence of a mix of amino acids namely serine, arginine and glutamine.

Antimicrobial activity

Antibacterial activities of the fresh and lyophilized cow urine samples were tested using disc diffusion method (Figure 2). The zone of inhibition as measured by disc diffusion assay is presented in Table 2.

Discussion

Commonly, antibiotics are used widely as a conservative treatment in various microbial infections and diseases. Most of these bacteria acquire resistance to these antibiotics and in order to battle these problems many alternatives are being explored. The ancient literature of cow urine has always been paid attention on prevention and treatment of diseases and maintenance of the health.

Cow urine has been found to be enriched with minerals, vitamins, urinary proteins, phenols and many other volatile and non-volatile compounds which may contribute to its antimicrobial nature [17]. The presence of protein in the samples was of clear evidence that all the samples do contain the bioactive compounds.

Naturally occurring peptides can be used as an alternative to chemical antibiotics and also as an antimicrobial substances [18]. The chromatography of the sample revealed the presence of the amino acid. It is better to remove urea and desalt the urine before going for paper chromatography and also not a single

Table 2. Zone of inhibition (in mm)

Sl. No.	Bacterial Pathogen	Zone of Inhibition (mm)**	
		Fresh cow urine	Lyophilized cow urine
1.	*E. coli*	14.67±0.57	18.67±0.88
2.	*Salmonella typhimurium*	16.66±0.33	19.33±0.33

**Values are mean±SE of three readings

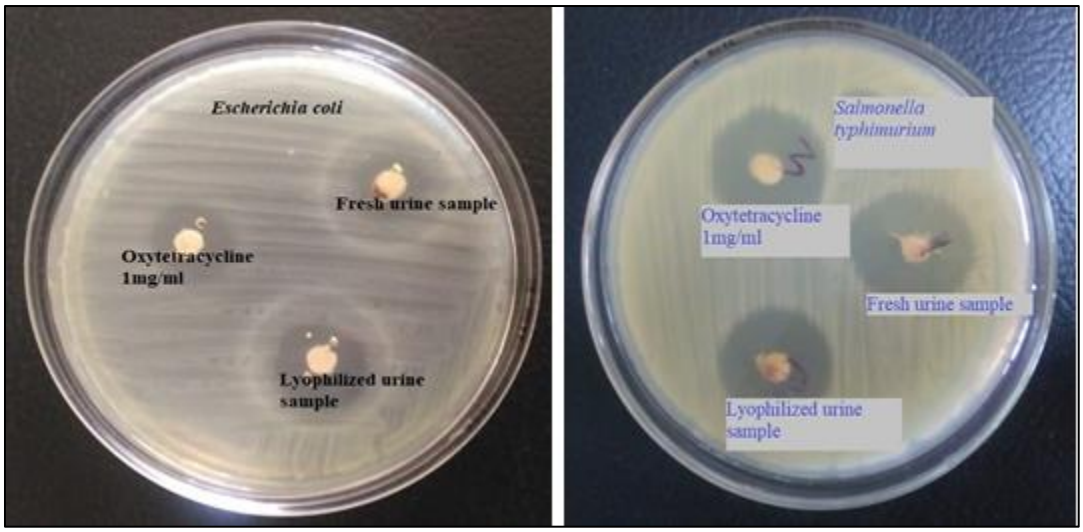

Figure 2. Disc diffusion assay

method can separate all amino compounds of urine [19]. The presence of amino acids may be responsible for contributing to the bactericidal effect as it increases the hydrophobicity of cell surface [20].

Antioxidant property of cow urine may provide potential therapeutic in intervention against oxidative threats, both in health and disease. The result suggests that the antioxidant action is attributed to the free radical scavenging activity of the cow's urine components. Fresh cow urine could be a potential source of natural antioxidant [5]. Fresh cow urine had better antioxidant activity compared to residue, distillate and re-distillate [21].

Gram negative bacteria were efficiently inhibited by the cow urine. The bacterial activity in the present study also coincides with the reports showing potential antimicrobial activity in fresh cow urine. Lyophilized cow urine has shown better activity compared to the fresh cow urine. The antibacterial activity of cow urine against the Gram's negative pathogens was nearly similar to the oxytetracycline. Photoactivated cow urine possess better antimicrobial activity compared to fresh urine possibly due to formation of some inhibitor substances namely ketones, formaldehyde and amines [22]. The mean zone of inhibition of fresh cow urine against *E. coli* was found to be 16 mm [23] indicating good antibacterial activity. The zone of inhibition as measured by disc diffusion method against *Salmonella* was 21 mm in photoactivated urine [24]. The ability of cow urine to inhibit the growth of microbes may be attributed to the peptides and proteins present in it.

Conclusion

From the above study, it can be concluded that the cow urine may contain bioactive peptides which collaborates with its antimicrobial action and it also possess a good antioxidant value.

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