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Evaluation of Antibacterial and Antioxidant Properties of Indigenous Cow Urine

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ABSTRACT

The article was emphasized on evaluation ofantibacterial and antioxidant potential of indigenous cow urine.Cow urine collected from rural area undertaken for analysis to establish antibacterial and antioxidant activity using agar well diffusion as well as DPPH assay. The zone of inhibition against test bacterial strains and DPPH assay hasrevealed promising results which confirms that the cow urine as a potent therapeutic agent. The presence of lipase enzyme in urine makes it highly potential anticancer agent which can be detectedby performing thin layer chromatography (TLC) and titrimetric method.

Keywords: Cow urine, antibacterial activity, antioxidant activity etc.

I. INTRODUCTION

The medicinal importance of cow urine (Gomutra) is well described in Ayurveda. Cowpathy is a treatment (in Ayurveda medicine) based on products obtained from cows called Panchagavya[1]. The use of Panchgavya in preparing medicinal and agricultural products are effective, eco-friendly and free form toxic effect to mankind. Recently cow's urine is being used as an effective medicine under cowpathy which is capable of curing blood pressure, thyroid, blockage in arteries, asthma, constipation, diabetes respiratory diseaseand certain types of cancer [2-5].

Cow urine contain N, S, Fe, Si, Cl, Mg, Na, citric salt, succinic salt, calcium salt, vitamin A, B, C, D, and E, lactose, creatinine, harmones ,urea and enzymes. It heightens the fact that cow urine is free from toxicity and contains 95% of water, 2.5% urea and remaining 2.5% a mixture of salts, hormones, vitamins and enzymes [6]. The main component in urine is urea known as micro-organismskilling agent thus is used as effective antiseptic for skin diseases, wounds andbeside this enzymatic action of urine can cure the various diseases including cancer [7, 8]. Cow urine enhances secretion of interleukin-1 and interleukin-2, as well as phagocytic activity of microphages and thus helps in the control and prevention of infections [9].Nowadays, different preparation of cow urine like photoactivated urine, urine distillate and fresh urine have been marketed as a reedy to get rid of various infections[10].

Cow urine is believed to have used in many drug formulations and also as a radical scavenging activity. The free radicals in our body damage the cell and its cellular components therefore it causes genetic disease.

Antioxidants are the substances that scavenge these harmful free radicals and prevent the damage to the cellular components [11]. The cow urine and its distillate have the ability to prevent, delay or ameliorate many of the effects of free radicals [12] and thus show antioxidant activity [13].These properties of indigenous cow urine develop our interest to study antibacterial activity against different pathogenic human bacterial strains and antioxidant activity.

II. MATERIALS AND METHODS

Urine extract collected early in the morning about 5:00 am as first urine micturition from local cow of urban area of Akola district situated in Maharashtra. The sample was filtered and stored in refrigerator in closed sterilized container preventing light oxidation. The boiling point of sample was recorded using digital melting point apparatus (Veego DMP) and is uncorrected.

The antimicrobial activity also investigated against some selected pathogenic human bacterial strains using agar-well diffusion method. The zone of inhibition recorded in mm and compared with streptomycin as a standard. The DPPH free radical scavenging activity of cow urine also carried out at 517nm. The lipase enzyme detection was done by performing thin layer chromatography using ninhydrin spraying agent and titrimetric method.

Antimicrobial Activity

The antibacterial action of cow urine against selected pathogenic bacterial strains such as *E. coli, K. pneumonia, P. aerugionasa, S. aureus and B. subtilis* was performed by agar well diffusion method.[9 x14]. The 20 ml of sterile Muller Hinton agar was poured in sterile petri plates and allow solidify. The 8 mm wells were made using sterile borer and 100µg of cow urine sample was micropippetted into wells. The zones of inhibition were recorded in mm after incubation for 24 h at 37^{0}C. The inhibition zone of the urine sample compared with the streptomycin as a standard reference.

Table 1: Antibacterial activity of cow urine against selected pathogenic bacterial strains

Bacterial strain	**Zone of inhibition in mm**		
	Fresh urine	**Urine distillate**	**Streptomycin**
E. coli	16	12	16
Klebsiella pneumonia	15	11	17
Pseudomonas aeruginosa	17	13	19
Bacillus subtilis	20	15	29
Staphylococcus aureus	18	13	26

Antioxidant Activity

The free radical scavenging activity 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 urine 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.

$$\%\ \textbf{Scavenging of DPPH radical} = \frac{[(Acontrol\ -\ Asample)]}{Acontrol}\ 100$$

Table 2: The DPPH free radical scavenging activity urine samples (µg/ml).

	% Scavenging of DPPH at 517nm					
	100	200	400	600	800	1000
Fresh cow urine	18.3±0.14	28.8±0.27	46.2±0.10	54.8±0.16	58.20±0.10	68.4±0.3

Detection of Lipase Activity

1) Titrimetric Method

A mixture of olive oil emulsion (5ml), tris hydrochloride buffer (5ml) at PH-8.5 and urine samples (2ml) incubated at 35^{0}C for 20 mins. After incubation addition of acetone (10ml), free fatty acid liberated in the reaction mixture was titrated against 0.05 M NaoH using phenolphthalein as an indicator. The blank titration was performed, in order to calculate lipase activity in a unit.One unit lipase activity was defined as amount of enzyme liberated 1 mole of fatty acid per minute.

Similar experiment was carried by addingurine sample and repeated two times to get constant readings. Formula for calculating lipase activity is given below.

$$\textbf{Lipase enzyme} = \frac{[\boldsymbol{Exp.titration\ reading}\ (\textbf{ml}) - \boldsymbol{Blank\ titration\ reading}\ (\textbf{ml})]\ \textbf{x}\ \boldsymbol{Molarity\ of\ NaOH}\ \textbf{x 1000 x 2}}{\boldsymbol{Volume\ of\ test\ urine\ sample}}$$

2) Thin Layer Chromatography

The cow urine sample was spotted on this slides of silica-gel-G-plates were prepared by using silica gel of thick broth on clean glass slides andimmersed in developing solvent comprising of chloroform and acetic acid (ml) in proportion of 4:1. Slide wasremoves after 30-40 min. and sprayed with ninhydrin solution, dried it using hot air oven.The pink color spot was observed on the plates indicated the presence of proteins which confirmed the presence of lipase enzyme.

a) Volumetric analysis b) Thin layer chromatography

III. CONCLUSION

From the above study, it can be concluded that cow urine exhibit remarkable antibacterial activity against selected pathogenic bacteria which helps further studies of bioactive component of cow urine which may address to unmet therapeutic needs. Cow urine may showlipase enzymatic action which collaborates with its antimicrobial action as well as good antioxidant activity.

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