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Review Article

COMPARATIVE ANALYSIS AND ANTIMICROBIAL STUDY OF COW'S URINE FROM RURAL AND URBAN AREAS

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ABSTRACT

Ayurveda stated importance of cow's urine for all purposes including in our daily life. This article was an emphasis on chemical content of urine which could make it potential factor for its usage as a medicine. A sample collected from rural area and urban area undertaken for biochemical analysis, for the comparative study of their antimicrobial activity. The presence of lipase enzyme in urine makes it highly potential anticancer agent. Detection of lipase activity of urine was done by performing thin layer chromatography, titrimetric, and colorimetric analysis.

Keywords: Ayurveda, Cow's urine, Lipase, Antimicrobial activity.

INTRODUCTION

Ancient Ayurveda literature reveals that cowpathy is a treatment (in Ayurveda medicine) based on products obtained from cows [1]. Recently cow's urine is being used as an effective medicine under cowpathy which is capable (of curing various) diseases including certain types of cancer [2]. Cow's urine contains 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's urine is free from toxicity (effluent) and contains 95% of water, 2.5% urea, and remaining 2.5% a mixture of salts, hormones, vitamins and enzymes [3]. The key component in urine is urea, which is known to kill micro-organisms. In rural village areas in India, cow's urine is used as effective antiseptic/disinfectant and for purification, for skin diseases, wounds, etc. the current research proves that enzymatic activity of urine can cure the various diseases including cancer [4,5]. Cow's urine augments B and T-lymphocyte blastogenesis and IgA, IgG and IgM antibody in mice. It also enhances secretion of interleukin-1 and interleukin-2, as well as the phagocytic activity of microphages and thus helps in the control and prevention of infections [6]. Cow's urine is believed to have therapeutics value and used in many drug formulations. It has also been reported that cow's urine is capable of curing blood pressure, blockages in arteries, diabetes, heart attacks, asthma, piles, migraine, ulcer, and gynecological problems. These properties develop our interest in comparative analysis and antimicrobial activity of cow's urine in rural and urban areas (in Akola district of Maharashtra).

METHODS

Urine extract from local cows of the urban area (4:00 am) and rural area (5:00 am) of Akola district situated in Maharashtra was collected early in the morning as first urine micturation. These Sample A (rural) and B (urban) are filtered and stored in a refrigerator in closed sterilized container preventing light oxidation. The boiling points of cow's urine A and B were recorded using digital melting point apparatus (Veego DMP) and are uncorrected.

Biochemical analysis for lipase detection was done by performing thin layer chromatography (TLC), titrimetric, and colorimetric analysis. The antimicrobial activity also investigated against some selected micro-organisms.

TITRIMETRIC ANALYSIS FOR DETECTION OF LIPASE ACTIVITY

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

All experiments were carried for Sample A and B and repeated two times to get constant readings. Formula for calculating lipase activity is given below.

$$\text{Lipase activity} = \frac{\left[\begin{array}{l}\text{Experimental titration reading in ml} \\ -\ \text{Blank titration reading in ml}\end{array}\right] \times \text{Molarity of NaOH} \times 1000 \times 2}{\text{Volume of test sample (urine A or B)}}$$

This titration value confirms the more concentration of lipase enzyme in Sample A than Sample B (Fig. 1).

ANTIMICROBIAL ACTIVITY

In the well-diffusion method, 100 µg of cow's urine sample was micropipetted into wells within the nutrient agar [7]. The zones of inhibition were recorded in mm after incubation for 24 hrs at 37°C. Inhibition zone record of the compounds indicated that Sample A and B were highly active against *Escherichia coli* and *Staphylococcus aureus*. To determine minimum inhibitory concentration, the serial dilution technique was followed using nutrient broth medium.

E. coli was more sensitive against Sample A (rural) than Sample B (urban), and *S. aureus* was more sensitive against Sample A (rural) than Sample B (urban) (Fig. 2).

TLC FOR DETECTION OF ENZYME IN COW URINES

Silica-gel-G-plates were prepared using silica gel of thick broth on clean glass slides.cow urine Sample A and B were spotted on this slides and immersed in developing solvent comprising chloroform and acetic acid in a proportion of 4:1. Slide A and B were took after 30-40 minutes and sprayed with ninhydrin solution, dried it using hot air oven.

The pink color spot was observed on both the plates indicated the presence of proteins which confirmed the presence of the enzyme.

On spraying ninhydrin solution, darker pink spots were observed on Slide A than on Slide B, which confirmed the higher concentration of lipase in Sample A (rural) (Fig. 3).

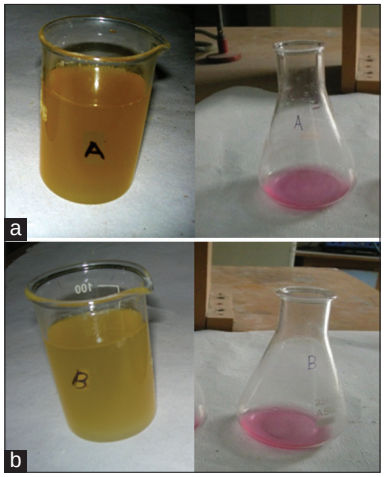
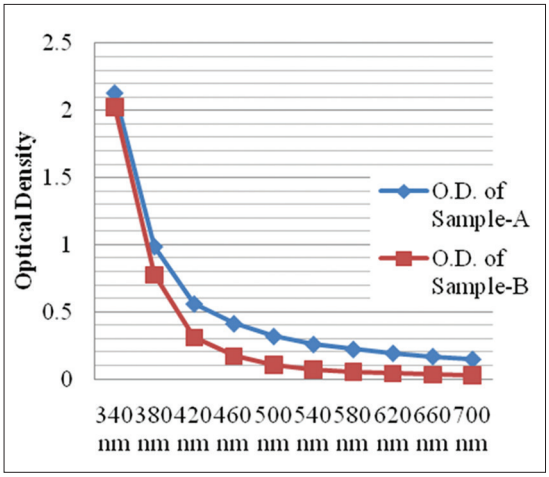


Fig. 1: Sample A and B were visualized colour change during titration for lipase enzyme



Graph 1: Callibration curves of samples plotted between wavelength and optical density

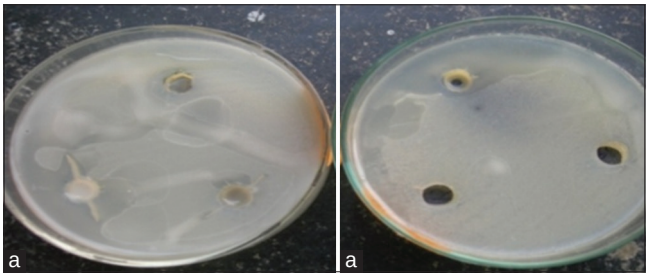


Fig. 2: (a) *Escherichia coli* against Sample A and B, (b) *Staphylococcus aureus* against Sample A and B

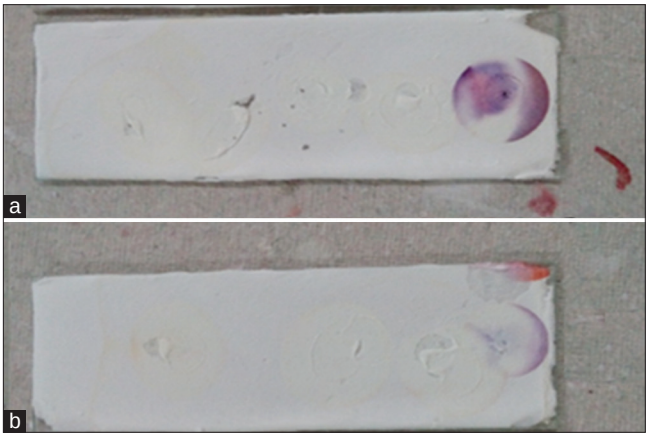


Fig. 3: Thin layer chromatography for sample A and B for detection of lipase enzyme

COLORIMETRIC ANALYSIS OF COW'S URINE

The cow urine Samples A and B were diluted by taking 1 µl in 3 ml distilled water. Centrifuged the diluted samples at 1000 rpm. The supernatant from these samples was analyzed on colorimeter at 200-700 nm. Graph 1 plotted optical density of Sample A and B against wavelength in nm.

CONCLUSION

The experimental study proves that cow's urine of rural area is one of the best sources of lipase enzyme. From the biochemical analysis, for the comparative study of the antimicrobial activity of sample collected from rural (A) and urban (B) clearly reveals that cow's urine sample collected from rural area was highly effective in inhibiting or controlling the growth of various micro-organisms than the sample collected from the urban area. Detection of lipase was performed by TLC, titrimetric, and colorimetric analysis and antimicrobial activity of urine was performed by cup plate diffusion method indicates the presence of lipase enzyme found to be more in rural urine sample make it highly potential anticancer agent.

Table 1: Optical density data of sample A and B by colorimetric analysis

Wavelength in nm	OD of Sample A	OD of Sample B
340	2.024	2.128
380	0.773	0.985
420	0.307	0.558
460	0.168	0.411
500	0.101	0.316
540	0.067	0.257
580	0.049	0.220
620	0.038	0.191
660	0.030	0.167
700	0.023	0.146

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