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Cow's Urine: An Incredible Aqueous Phase

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Abstract: The study was carried out biochemical analysis of outdoor grazing photo activated cow's urine and explored antimicrobial activity thereof. Urine sample was collected from adult outdoor grazing dwellers and was subjected to process of photo activation. It was analysed to physical properties, organoleptic tests, viscosity, *ex-vivo* studies, UV scan, TLC, Milk test, volumetric analysis and skin irritation test. It had almost same physical properties similar to that water; milk test and TLC studies proved presence of lipase enzyme. There was absorption in UV range due to lipase enzyme, *ex-vivo* studies showed high zone of inhibition for antimicrobial agent with respect to water and outdoor grazing photo activated cow's urine also ($P<0.05$). Study confirmed that outdoor grazing photo activated cow's urine had additive antimicrobial activity for antimicrobial agents due to lipase enzyme and improvement in MIC values thereof.

Key words: Cow's urine · Antimicrobial activity · Lipase enzyme and *ex-vivo* studies

INTRODUCTION

Cowpathy is a treatment by materials derived from cow. Cow's urine is execratory product of cow. It is advised as a substitute preventive and treatment system. Its various characteristics have been earned respect of the clinicians. Cow urine possesses 95% water, 2.5% urea; minerals and various salts; 2.5% hormones and enzymes (Table I).

Fig. 1: OGPhCU sample collected in late evening

Cow urine kills a number of drug resistant microbes. As a bio enhancer it increases the efficacy of antimicrobial agents. This potential has been observed to reduce dose and dosage too. In short cow urine is modern version of old science. [1-3] Cow urine administration paralleled to antimicrobial agents delay emergence of resistance. Due to the presence of quinolones & quinolones derivatives, it possesses significant fungistatic and fungicidal properties. Cow urine is useful for many fungal agents [4]. Only earthen pot or glass pot must be used for preparation of Cow's Urine distillate. First boil cow urine so that ammonia gas is removed simultaneously odour would be gone away. The vapour of cow urine is to be collected by tube device like in distillation process. This is called Cow's Urine distillate [5]. Cow urine distillate also has a bio enhancer action without odour. It enhances the transport across gut wall and bioavailability of antibiotics by several folds [6-8]. The bio enhancing property is by pertaining absorption through membrane of cell [9]. In *Auyrveda*, OGPhCU was prescribed as a vehicle with broad spectrum antibiotic effect, [10] however the wonderful thing observed

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that factors such as storage do not affect potential or activity of cow's urine distillate. It has longer shelf life and its biochemical activity never declines. It acts as an effective antifungal agent againsteven *Candida albicans*. Specimen from outdoor grazing cow was more vital than indoor feeding [5, 11-13].

Table I: Chemical constituents of cow's urine & their Therapeutic uses

Chemical constituents	Therapeutic uses
Water more than 95%	aqueous phase
Na, N and S; Retinol, Vit. B, E, Ascorbic acid, calcitriol	Vitamin supplements
minerals, manganese, iron, silicon, chlorine and magnesium	Blood purification
phosphate, lactose, carbolic acid and enzymes	Pepsin stimulant
Citrate, Succinate, Calcium salts	Anti-ageing agent
Creatinine	To maintain bilirubine concentration
Hormones	Bio enhancer by way of changing bacterial cell surface hydrophobicity and decreases drug resistance and by blocking the R-factor.
Urea, uric acid, minerals, salts	Antibacterial, improves phagocytic activity of macrophages.
Urea	Penetration enhancer, Keratolytic
Lipase	fat destroyer

Table II: Quantitative Analysis of Chemical constituents of cow's urine

Chemical Constituents	Per Kg/Day
Urine volume	17-45ml
Urea nitrogen	23-28ml
Ammonia nitrogen	1-1.7ml
Total nitrogen	40-45ml
Allantoin	20-60 ml
Calcium	0.1-1.4 ml
Chloride	0.1-1.1 m mol
Coproporphyrin	5-14µm/dl
Creatinine	15-20 mg
Mg	3-7 mg
K	0.08-0.15 mmol
Na	0.2-1.1 mmol
SO_4	3-5 mg
Uric acid	1-4 mg
Uroporphyrin	1.5-7 mg/dl
Leucocyte	Less than 15 µl

Side effects of cow's urine therapy were observed like itching, bad smell etc. Sometimes antibacterial property of photo activated cow urine was very much at a very low concentration. If OGPhCU was mixed with neem oil than MIC value would be higher than that of antibacterial agent itself this is so because of traces of volatile organic materials present in urine which would become severe toxic due to photo activation [14-16]. Several studies also concluded that sample collected in early morning was highly effective than that of any other time because early morning sample has a comparatively higher content which were responsible for antimicrobial activity (Table II) [17].

MATERIALS AND METHODS

Urine of Cow was supplied from Punit *Gaushala* Rajkot, India, Salicylic acid purchased from Sewa fine Chem. Ahmedabad, Ciprofloxacin purchased from Mark Spl. Pvt. Ltd. Mumbai and Chloramphenicol purchased from Oxford Laboratory Thane. The test organism used in this study was Blossom™ Dry yeast (*Saccharomyces cerevisiae) and Staphylococcus aureus* procured from the School of Science, RK University Rajkot, India. Specific gravity bottle and Oswald's viscometer purchased from Borosil® (I) Pvt. Ltd. Olive oil, Tris-Hydrochloride (Tris-HCl) buffer, Concentrated Hydrochloric acid, Sodium hydroxide flakes, phenolphthalein, Ninhidryin etc. were purchased from Astron Chemicals ltd, Ahmedabad, India.

Collection and Purification of Cow Urine: Breed of cow (*Bos taurus indicus*) with more than 4 years of age was chosen. Collected urine was subjected to photo activate for 3-6 days in sunlight. It was filtered and stored in cool and dry place [14].

Evaluation of Physical Properties: Organoleptic Tests: Photo activated cow's urine was inspected for odour, colour, taste, homogeneity, optical clarity and fluidity [18].

Viscosity: Oswald's viscometer was used to measure viscosity at room temperature. The equation 1 was used to measure viscosity:

$$\acute{\eta}1 = \frac{d1t1}{d2t2}\acute{\eta}2 \qquad \text{Equation 1}$$

where,
ή1=viscosity of sample
ή2=viscosity of water=0.845cp

d1=density of sample
d2=density of water=1.004 gm/ml
t1=time from A to B in Viscometer for sample
t2= time from A to B in Viscometer for water [19]
Density: The specific gravity of the systems was determined at ambient conditions using a specific gravity bottle of Borosil® glass of 10 ml capacity.
pH: pH was measured using digital pH meter of Shimadzu Japan.

UV Spectrophotometric Analysis: OGPhCU was diluted in manner of 0.1 ml in 300 ml of water and centrifuged at 500 rpm for 50 min; one at room temp (Indian summer morning condition 27 ± 2°C) and one at elevated temperature or at 55 ± 5°C then allow to cool at room temperature. These sample were analyzed by double beam UV Spectrophotometer, model no. LT-2900, of Labtronics (I) Pvt. Ltd., Ambala, at 200 nm to 400 nm keeping cool water as blank [17, 20]. Solubility was also measured by means of super saturation method at 25°C ± 2°C by the Spectrophotometry and simultaneous method of spectroscopy for quantitative detection. Unpaired Student's t test with equal variance could be used to find any statistically significant difference in the solubility of antimicrobial agent between water and OGPhCU at 5% level of significance [21, 22].

***Ex-vivo* Study:** *Ex-vivo* study was carried out by well diffusion method. Blossom™ Dry yeast was taken in test tube filled with hot water add slight sugar and NaCl to it, put in dark for few minutes. This was taken as culture for *Saccharomyces cerevisiae* [23, 24]. Subrogated dextrose agar and Nutrient agar broth medium plates were made, sterilized by 15 psi, 120°C for 90 min and 1 ml of prepared baker's yeast and *Staphylococcus aureus* (MTCC-3160) cultures was inoculated in these plates respectively. After solidification, boring was done and samples of salicylic acid in urine in Subrogated dextrose agar and ciprofloxacin and chloramphenicol in urine in Nutrient agar broth medium were poured into it by micropipette (100 µl). These were incubated in BOD at 37°C for 1 day and subjected to observation. [25, 26] Unpaired Student's t test with equal variance could be used to find any statistically significant difference between zone of inhibition of antimicrobial agent between in water and in OGPhCU at 5% level of significance.

TLC Analysis: Prepare 25% Silica gel slides and used for study. Readymade chromatographic papers were also used. Sample drop was allowed to run on one the edge of plate and then these were gently put in running solvent mixture of chloroform: acetic acid (4:1). This assembly was put a side for 1 hr for saturation and then remove plates from assembly and sprayed with ninhydrin solution and put a side for drying [17].

Milk Test: 2-3 ml of urine added to milk taken in a glass cylindrical cup and subjected for visual inspection [17].

Volumetric Analysis: In one flask add 5ml of olive oil, 5ml of 0.1 M Tris-Hydrochloride buffer of pH 8.5 and 1.0 ml of OGPhCU at 27°C for 20 ± 2 min. This system was allowed to centrifuged at 150 rpm, After incubation then add 10 ml of acetone, add a few drops of phenolphthalein and titrated with 0.05 N NaOH until pale pink colour was obtained. The blank assay was also done by adding acetone solution to flask immediately without allowing for incubation for 20 min.

The lipase enzyme was calculated as equation 2:

$$\text{Lipase enzyme (in units)} = \frac{[(\text{Test Value} - \text{blank value}) X \text{Molarity of NaOH} X 1000 X 2 X DF]}{\text{ml of the Sample}} \quad \text{Equation 2}$$

where, DF=1 [17].

Skin Irritation Test: Approval to carry out *in-vivo* studies was obtained from the Institutional research Ethics Committee (EC) from CDSCO, Director General of Health Services, ministry of Health & Family Welfare, Govt. of India at RK University, Rajkot, Gujarat, India and their guidelines were followed were in accordance with the Declaration of Helsinki (1975, amended 2013) on experimentation on human subjects. Twice a day for 7 days of OGPhCU was applied on human volunteer and site was observed for any sensitivity and reaction. The sensitivity was graded as 0, 1, 2, 3 and 4, for non-irritant, mild-irritant (slight pink), moderately irritant (dark pink), moderate to severe irritant (light red) and severe irritant (extreme redness) with or without inflammation, respectively [27].

RESULTS

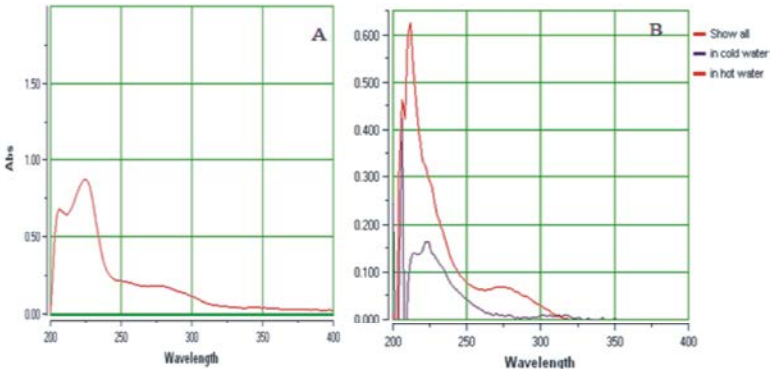

Fig. 2: Scanning of OGPhCU under 200-400 nm (A) in methanol (B) in distilled water at room temperature and at elevated temperature

Table III: Physical Properties

Properties	Result
Colour	Yellowish brown
pH	7 ± 0.5
Density (gm/ml)	1.03 ± 0.1
Viscosity (cP)	2 ± 0.1
Taste	salty & bitter
Shelf-life	5 years
Homogeneity	Good

mean ± SD; n=3

Table IV: Solubility studies

	Solubility (mg/gm) at 30°C	
Antimicrobial agent	Water	OGPhCU
Salicylic acid	2.48 ± 0.06	2.34 ± 0.05
Ciprofloxacin	0.53 ± 0.01	0.528 ± 0.006
Chloramphenicol	2.5 ± 0.05	2.5 ± 0.08

mean ± SD; n=5

Fig. 3: TLC slides sprayed with ninhidryin solution (first photograph of readymade chromatographic paper and the other three photographs are of prepared silica gel slides)

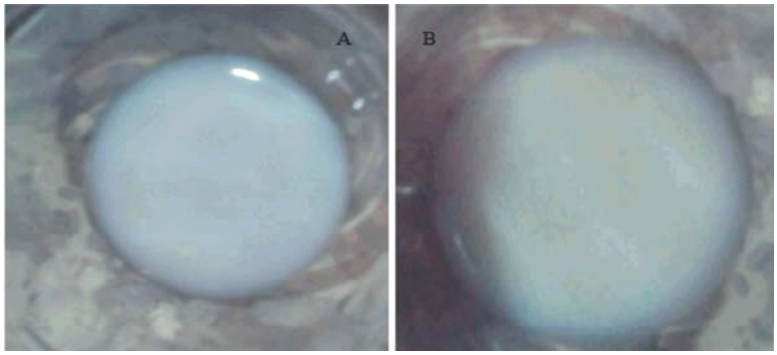

Fig. 4: Milk test (A) before adding of OGPhCU (B) after adding of OGPhCU

Table V: Volumetric Analysis

Sample	Avg. Value of titre
Test	2.48 ± 0.104
Blank	2.0 ± 0.079
Lipase enzyme (units)	48 ± 2.5

n=5; Mean ± SD

Fig. 5: Titration end point pink shade

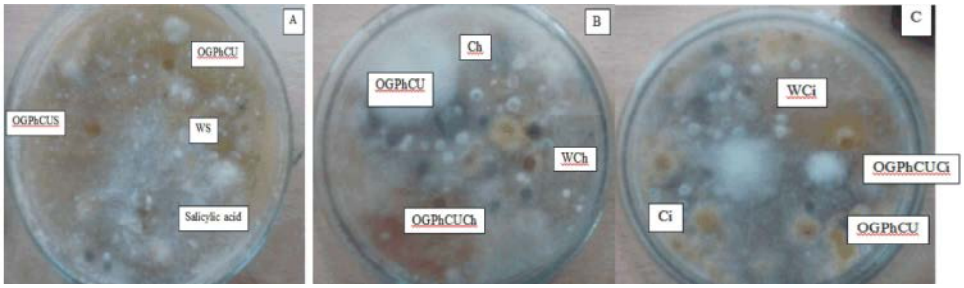

Fig. 6: *Ex-vivo* study (A) Salicylic acid for *saccharomyces cerevisiae* (B) Chloramphenicol for *Staphylococcus aureus* (C) Ciprofloxacin for *saccharomyces cerevisiae*

Table VI: % zone of inhibition for different specimens for salicylic acid

	Specimen	% Zone of Inhibition
Antimicrobial agent alone	Salicylic acid(S)	6.11 ± 0.878
	Chloramphenicol(Ch)	3.33 ± 1.76
	Ciprofloxacin(Ci)	5.56 ± 0.79
Antimicrobial agent in water	WS	7.67 ± 0.72
	WCh	7.78 ± 1.56
	WCi	6.45 ± 0.5
OGPhCU sample	OGPhCU for salicylic acid	12.22 ± 1.76
	OGPhCU for chloramphenicol	4.22 ± 2.08
	OGPhCU for ciprofloxacin	4.6 ± 2.09
Antimicrobial agent in OGPhCU sample	OGPhCUS	16.78 ± 1.94
	OGPhCUCh	16.89 ± 3.72
	OGPhCUCi	17.33 ± 2.56

n=5; Mean ± SD

DISCUSSION

Organoleptic Tests: OGPhCU was yellowish brown in colour due to traces of bilirubin and biliverdin present in it. (Figure 1), possessed unpleasant odour due to free ammonia present in it, pH slightly basic and slightly bitter taste because of nitrogenous materials present in it, density was 1.03 gm/ml and viscosity was 2 ± 0.1 because more than 95% of water content, even 5% of solid matter, homogeneity was good. (Table III)

UV Spectrophotometric Analysis: OGPhCU was possessed absorption spectrum in 200-400 nm range at 225 nm due to lipase enzyme present in it. (Figure 2A), lipase enzyme was solubilized partially in water upon heating, so peak became more sharpen when it was scanned in hot water. (Figure 2B)

TLC Analysis: The purple colour observed on paper chromatographic and pink on prepared slides as well, it was suggested that there was presence of an enzyme. (Figure 3)

Milk Test: There was incomplete clear zone on addition of OGPhCU in milk, which suggested that there could be absence of protease enzyme. (Figure 4).

Solubility: Solubility of salicylic acid, ciprofloxacin and chloramphenicol in OGPhCU almost near to thereof in water because more than 95% of OGPhCU was water (table IV). Pooled degree of freedom=5+5-2=8, tabulated t value at 5% level of significance is 2.31. Calculated t value was lower than tabulated. P>0.05. It can occur more than 5 times in 100 i.e. very frequent, hence less significant (t=0.1, 0.02, 0.1 respectively for salicylic acid, ciprofloxacin and chloramphenicol P>0.05 not significant at 5%), thus study was concluded that change of OGPhCU as medium from water there was no increased in the solubility of salicylic acid, ciprofloxacin or chloramphenicol.

Volumetric Analysis: lipase enzyme was found to be 48 ± 2.5 units per ml (Table V and figure 5). Any value of lipase would be able to provide lipase activity i.e. for digestion of fats.

***Ex-vivo* Studies:** zone of inhibition were higher for salicylic acid, ciprofloxacin and chloramphenicol with OGPhCU (Figure 6; Table VI) confirmed that it had concentration dependent antimicrobial activities, i.e. increasing volume of OGPhCU and antimicrobial agent, zone of inhibition increased and vice versa. Pooled degree of freedom=5+5-2=8, tabulated t value at 5% level of significance is 2.31. Calculated t value was higher than tabulated. P<0.05. It can occur less than 5 times in 100 i.e. very rare, hence more significant (t=4.82 and 5.13 respectively for salicylic acid and chloramphenicol and P<0.05 significant at 5%) and for ciprofloxacin tabulated t value at 10% level of significance is 1.86, calculates t-value was 2.04, it can occur less than 10 times in 100 i.e. rare, hence significant (t=2.04 and P<0.1 significant at 10%), thus study was concluded that change of OGPhCU as medium from water there was increased in zone of inhibition for salicylic acid, ciprofloxacin and chloramphenicol. This was so because of improvement of MIC values of salicylic acid, ciprofloxacin and chloramphenicol. OGPhCU could be bio enhancer and improved deposition of antimicrobial agent on cell-wall.

Skin Irritation: There were no signs of skin irritation (value '0') and inflammation or oedema, this was so because more than 95% of OGPhCU portion was water.

CONCLUSION

Studies were concluded that OGPhCU had physical properties almost like water with antimicrobial activity. It could be substituted with aqueous phase present in formulations useful for skin diseases while there would be needed to carry out more toxicity studies, *in-vitro* analysis, compatibility studies, patients' compliance, stability studies etc. to authenticate research prediction, so by this way there would be additive pharmacological action of antimicrobial agent without making so much efforts.

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Abbreviations

UV: Ultra Violet, TLC: Thin Layer Chromatography, nm: nano metre, Na: Sodium, N: Nitrogen, S: Sulphur, Vit. Vitamin, ml: millilitre, mmol: milimole, Mg: Magnesium, K: Potasium, SO4: Sulphate, rpm: Revolution per minute, NaCl: Sodium Chloride, BOD: Incubator, NaOH: Sodium hydroxide, DF: Dilution Factor, OGPhCU: Outdoor grassing Photo activated Cow's Urine, cP: Centi Poise, SD: Standard Deviation.

Author's Contributions: Mr. Kalpesh C. Ashara carried out the study and analyzed data; Dr. Saneesh Kumar and Mr. Dhaval A. Nirmal were participated in the design of the study and edited the manuscript. Dr. Ketan V. Shah was research guide. All authors read and approved the final manuscript.

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