



## Research Article

### ISOLATION, PURIFICATION AND PARTIAL CHARACTERIZATION OF UROKINASE FROM COW URINE OF INDIAN ORIGIN (*BOS INDICUS*)

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#### ABSTRACT

In Ayurveda, there are many medicines made from cow urine (Goumutra). This purifies, and clears all blocks in body channels (shroto-shodhaka). It enhances the therapeutic actions of medicines taken along with it. Cow urine has a unique place in Ayurveda and has been described in many ancient manuscripts like Atharva Veda, Charaka Samhita, Rajni Ghantu, Vridhabhagabhatt, Amritasagar, Bhavaprakash, Sachitra Ayurveda, and Sushruta Samhita to be the most effective substance / secretion of animal origin with therapeutic values. It has been recognized as 'Water of Life' or 'Amrita'. In India, drinking of cow urine has been practiced for thousands of years. Cow Urine Treatment And Research Center, Indore, India and Go Vigyan Anusandhan Kendra, Nagpur, India has conducted a lot of research over the past few years and has reached the conclusion that it is capable of curing thrombo embolic diseases, diabetes, blood pressure, asthma, psoriasis, eczema, heart attack, blockage in arteries, fits, cancer, piles, prostrate, arthritis, migraine, thyroid, ulcer, acidity, constipation, gynecological problems, ear and nose problems and several other diseases. The present work focuses on the isolation, concentration, purification of urokinase from freshly collected samples of cow urine from cows of Indian origin (*Bos indicus*), its partial characterization by SDS-PAGE, TLC (Tryptic digest protein) and proteolytic activity by gelatin zymography. The dialyzed cow urine proteins showed band identical to standard urokinase by SDS-PAGE. The itching of gelatin by dialyzed cow urine also shows the proteolytic activity.

**Keywords:** Cow urine, Urokinase, SDS-PAGE, TLC.

#### INTRODUCTION

Cow, *Bos indicus* is a most valuable animal in all veda and it is called as the Mother of all. In India, cow is worshipped as 'Kamdhenu' i.e. the god who fulfils all desires since thousands of years. Different products obtained from cow are useful to mankind either in medicines, agriculture or religious purpose. Panchgavya (Five imp. products of cow viz. urine, milk, ghee, curd and dung) having medicinal and spiritual importance in India. All these five (Panchgavya) having medicinal properties and are used either single or in combination with herbal or minerals against many diseases<sup>1</sup>. An exhaustive reference of cow's urine having curative properties in skin diseases, especially leprosy, is referred in charak samhita. Furthermore, in treatment of falling body parts, discharging lymphs and organism infected organs, use of cow's urine has been recommended for bath, anointing and intake. Feeding of cow urine increased the feed intake in white legborn layers<sup>2</sup>. The present study was carried out to prepare cow urine distillate and to determine the fibrinolytic activity.

#### MATERIALS AND METHODS

##### Collection of Sample

Fresh cow urine sample of Indian origin (*Bos indicus*) was collected in clean container from cow farm (Matoshri Anusandhan Kendra), Pune, India. The collected sample was stored in a refrigerator for further study.

##### Physical properties of cow urine

The collected sample of cow urine was observed for physical properties i.e. color, odor, pH, specific gravity etc.

##### Purification of Urokinase from Cow urine Ammonium Sulfate Precipitation

The concentration of ammonium sulfate was optimized to 70-80 % of saturation to give maximum protein precipitation. All steps were carried out at 2-8°C. The precipitated proteins were centrifuged.

##### Dialysis

The protein pellets formed after centrifugation were re suspended in 10 ml of 0.1M Tris-HCl buffer, pH 7 and tied in the dialysis membrane in small pouches and were kept immersed in beaker containing 0.1M Tris-HCl buffer, pH 7 (1000 ml) and dialysis was carried out. The dialysis beakers were kept on magnetic stirrer so that continuous concentration gradient was maintained. The temperature was maintained at 2-8°C for the whole process<sup>3</sup>.

##### Protein Estimation

##### Protein estimation by Lowery Method

Protein was measured by the method of Lowry *et al.*, (1951) with bovine serum albumin (BSA) as the standard<sup>4</sup>. The concentration of protein during purification studies was calculated from the absorbance at 680 nm.

##### Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SDS-PAGE was carried out by modified Lammelli *et al.*<sup>6</sup> A low molecular weight marker (BSA) was used as reference proteins. Gel was stained with coomassie Brilliant Blue R-250 and de stained with a solution containing methanol: glacial acetic acid: distilled water (1:1:8) The SDS-PAGE was performed according to Laemmli using 12.5 % gel.<sup>5</sup>

### Tryptic digestion of dialyzed cow urine proteins

A tryptic digest is accomplished simply by taking a pure, whole protein and subjecting it to certain enzymatic activity that breaks down the protein into specific peptides and or peptide chains. Trypsin is a serine protease that specifically cleaves at the carboxylic side of lysine and arginine. Dissolve 1-10 mg of the target protein in 6M guanidine HCl (or 6-8 M urea), 50mM Tris-HCl (pH 8), 2-4 mM mercaptoethanol in a reaction volume of up to 1 ml. Heat at 95°C for 15-20 minutes or at least 60°C for 45-60 minutes. However, under no condition should less than 25 µl of be used. After denaturation, allow the reaction to cool and add 50 mM NH<sub>4</sub>CO<sub>3</sub> (pH 7.8) or 50 mM Tris-HCl, 1 mM CaCl<sub>2</sub> (pH 7.6), until the guanidine- HCl or urea concentration is below 1M.

### Estimation of protease activity

#### Gelatin Zymography

The concentrated dialyzed fractions of cow urine of showing the highest protein content were subjected to gelatin Zymography which is an electrophoretic technique, based on SDS-PAGE, that includes gelatin copolymerized with the polyacrylamide gel, for the detection of enzyme activity.

### RESULT AND DISCUSSION

The urine sample collected from cow was analyzed in laboratory condition for physical properties and total protein content

Table 1: Physical properties of cow urine

Color	light yellowish
Odor	Strong
pH	7.9
Specific gravity	1.031 g/ml

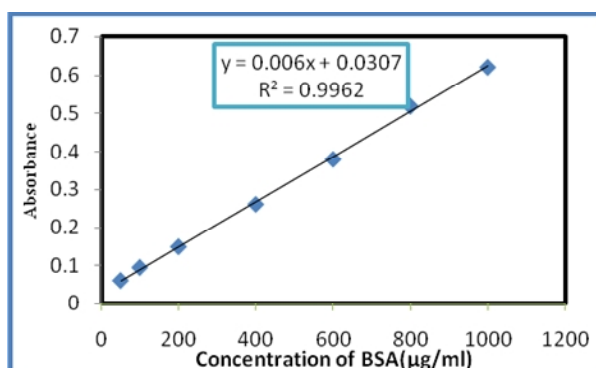


Figure 1: Standard Calibrations curve of Bovine serum albumin (BSA)

The precipitated protein fraction of cow urine was dialyzed in dialysis membrane for 24 h, after dialysis sample were tested for total protein content.

Table 2: Total protein estimation in dialyzed cow urine sample

S. No.	Batch Code	Total Protein (mg/ml)
1	CU1	5.7
2	CU2	4.8
3	CU3	5.2

\*CU- cow urine

The total protein content in concentrated protein fractions was found to be in the range of 5.7-4.8 mg/ml.

### Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis

The purified pooled urokinase fraction was analyzed by SDS-PAGE, the result observed consisted of a single polypeptide chain of high molecular weight urokinase with an apparent molecular weight of 59 kD band in Lane A. (Figure 2). The molecular mass standards were Phosphorylase b 97.4 kD BSA (66 kD), ovalbumin (43 kD), SDS-PAGE Lane B. The molecular weight was determined by interpolation from a linear semi logarithmic plot of relative molecular mass versus the R<sub>f</sub> value (relative mobility).

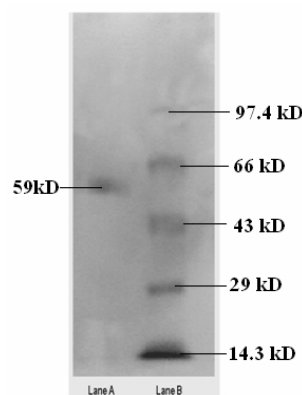


Figure 2: Characterization of Dialyzed cow urine sample by SDS- PAGE (12.5 %)

Lane A contains the 59 kD band of single chain high molecular weight urokinase chain taken from the pooled fraction. Lane B contains the medium molecular marker used as the standard on a 12.5 % SDS-PAGE.

### Tryptic digestion

Figure 3 shows the bands of digested dialyzed sample of cow urine sample and digested standard urokinase. Lane A and Lane B show the bands having relative R<sub>f</sub> value.

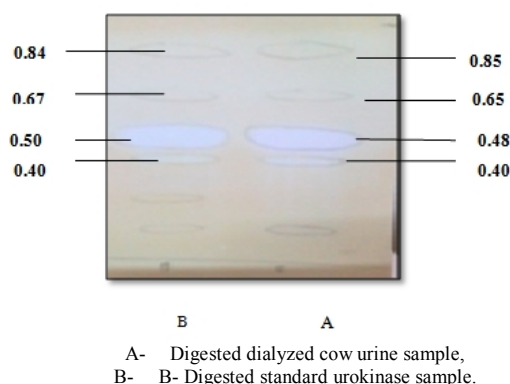


Figure 3: Characterization of Dialyzed cow urine sample by TLC, R<sub>f</sub> value -A) 0.40, 0.48, 0.65, 0.85, B) 0.40, 0.50, 0.67, 0.84

### Gelatin Zymography

To test the plasminogen activating potential of the dialyzed cow urine sample, Zymographic analysis of the sample was performed (Figure 4) using reference urokinase as the positive control. The dialyzed cow urine sample produced detectable zones of lysis on the Zymogram (Figure 4, Lane

A) in the molecular range of 59 kD which corresponded to the band observed in the SDS-PAGE pattern.



Figure 4: Gelatin Zymography showing the itching of gelatin

Zymogram of pooled peak fractions of dialyzed cow urine and standard urokinase, Protein (20 µg) was loaded in Lane A and B and electrophoresis carried out. Lanes: (A) pooled purified urokinase; (B) urokinase (U-FRAG). Clear zones of lysis indicate bands of urokinase.

#### CONCLUSION

We have made an attempt to investigate the presence of proteins in cow urine. The SDS-PAGE analysis of dialyzed cow urine sample shows band identical to the standard

urokinase. TLC of tryptic digest of dialyzed cow urine protein shows spots having the same  $R_f$  values with that of tryptic digest of urokinase standard.

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