

ISOLATION OF HIPPURIC ACID FROM BUFFALO URINE AND ITS ANTIOXIDANT ACTIVITY

S. Roobinisha¹, K. Elango¹, S. Jothimani¹ and R. Subramanian^{2,*}

ABSTRACT

Hippuric acid is a main component present in buffalo urine. Hippuric acid is a crystalline compound possess optical property. The aim of the present work is to isolate hippuric acid (HA) from the buffalo urine and evaluate its antioxidant activity. The hippuric acid crystals were isolated from the buffalo's urine by crystallization method and characterized using spectral techniques such as UV-Visible spectrophotometer (UV-Vis), Infrared spectrophotometer (IR) and X-ray diffraction studies (XRD). The antioxidant activity of HA was determined using 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH). The spectral characterization in comparison with the literature data confirmed the presence of hippuric acid. The antioxidant activity of HA was comparable with that of the gallic acid. It is concluded that buffalo urine is a natural source of HA and it could be explored for commercial purpose.

Keywords: buffalo urine, hippuric acid, crystallization, antioxidant activity

INTRODUCTION

Initially, Hippuric acid (HA) is the interest of Physician to find out its role in liver function about sixty decades ago. HA is one of the important amino acids synthesized in the liver as a metabolite of benzoic acid urinary excretion (Borsook and Dubnoff, 1939). It is proved that a rabbit could able to combine benzoic acid with glycine and glucuronic acid to form HA along with benzoyl glucuronide. It has been shown that the administration of glycine with the benzoic acid increases the rate at which HA is excreted by the kidneys (Hainline and Lewis, 1953). Hippuric acid had been separated from cow urine with some pigments by the method of precipitation followed by the addition of ammonium sulphate to the urine before acidification (Roaf, 1908). Later, Ringertz (1971) had established the structure of HA using X-ray diffraction study. HA had drawn the attention of researchers because of its optical property. It is formed in the order of orthorhombic structure. It is proved as good crystal for second harmonic generation because of its high conversion efficiency (Chemla and Zyss, 1987; Kumaresh and Kumar, 2014). Optical property of HA has been found by researchers as an advanced

¹Department of Chemistry, K.S.Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Tamil Nadu, India

²Department of Chemistry, Sun Arts and Science College, Tiruvannamalai, Tamil Nadu, India,

*E-mail: subu_m1@yahoo.com

study. Optical properties of hippuric acid caused by the presence of a donor like NH_2 and acceptor like COOH , and intermolecular charge transfer are also possible (Capllonch *et al.*, 2001). Some researchers have synthesized substituted hippuric acid crystal and evaluated their optical properties. For example, *p*-amino hippuric acid (Babu *et al.*, 2006), rhodamine dye doped hippuric acid (Sathesh *et al.*, 2014) and 4-amino hippuric acid (Rajaa *et al.*, 2014) have been synthesized and utilized for various applications. Due to the presence of donor atoms such as nitrogen and oxygen, hippuric acid can easily form complex with metals. Many researchers have proved that HA can react with metal ions such as Cd (II), Hg (II), Zn (II), Mn (II), Co (II), Ni (II), Ag (I) to form coordinate bonds through a carboxylic oxygen atom (Badawi and Al-Saadi, 2011).

In rural India, cow and buffalo are a part of farmers' life for Milk production which provide nominal support to the farmers. By-products such as dung and urine of the both cattle are used as fertilizer. Among the house animals, buffalo and cow gained more importance since ancient days due to its ritual. Cow urine is believed to have therapeutic value and used in many drug formulations. Traditionally, cow urine is used as disinfectant in India. Cow urine is a natural antiseptic and disinfectant. It is easily available in villages and environmentally friendly when compared with synthetic chemicals those are currently available to the consumers. In the rural area of India, cow urine is being used very long time as an effective antiseptic for wounds, skin diseases and bathing (Kumar, 2013). Cow urine proved to have some essential minerals, hormones and enzymes. Apart from that it contains necessary minerals, which enrich the fertility of soil (Bhadauria, 2002). The application of buffalo urine has not been explored

as compared with cow urine. Utilization of buffalo urine for the isolation of hippuric acid is a green process which minimize the utilization hazardous chemicals in the synthesis. Bulk quantity of hippuric acid can be isolated for commercial application. Due to the optical property, hippuric acid can be valid candidature for material research. Hence, the aim of the present work is to isolate hippuric acid from buffalo urine by crystallization methods and evaluate its antioxidant activity to test its bioactive potential.

MATERIALS AND METHODS

Sample collection and concentration

Urine (3.5 liter) was collected from three different Murrah buffalo's and mixed together in a cleaned container. The sample was thoroughly filtered using Whatman filter paper. The volume urine sample (3.5 liter) was reduced into 100 ml by distillation. Brownish liquid with solid mass remained in distillation flask was transferred into another container and allowed for settlement. Brown solid was separated from brownish liquid. Brown solid repetitively washed with cold water and kept aside. Then brown liquid was again filtered through Whatman filter paper and centrifuged at 5000 rpm for 10 minutes to remove the residual material.

Separation of hippuric acid by crystallization method

A clear brown liquid obtained by the above method was poured into 250 ml beaker and kept aside at room temperature without disturbance. After 10 days, very tiny crystals were started growing. Then the beaker was left for another 5 days, slow evaporation led to the formation

bigger size brownish crystals. Brownish crystals were dissolved in enough quantity of hot water to produce saturated solution. This solution was again kept aside at room temperature for 10 days. Colorless, transparent crystals were grown and separated carefully and stored for further study.

Determination of antioxidant activity

The antioxidant capacity of HA was studied by the evaluation of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Subramanian *et al.*, 2013). Various concentrations of HA (0.3 ml) were mixed with 2.7 ml of methanolic solution containing DPPH radical (6×10^{-5} mol/l). The mixture was shaken vigorously and left to stand for 60 minutes in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation.

$$\% \text{ RSA} = \left[\frac{(A_{\text{DPPH}} - A_s)}{A_{\text{DPPH}}} \right] \times 100$$

Where A_s is the absorbance of the solution when the sample extract is added at a particular level and A_{DPPH} is the absorbance of the DPPH radical solution. Gallic acid was used as standard.

Characterization

UV-Visible spectrum recorded using Wenstar-Labman 100B spectrophotometer. The FTIR spectrum was recorded using Perkin Elmer FT-IR spectrometer (KBr pellet technique) in the range 400 to 4000 cm^{-1} . The X-ray diffraction (XRD) pattern was recorded using Rigaku Mini Flex II powder X-ray diffractometer in the range between $20^\circ \leq 2\theta \leq 60^\circ$ with Cu K α monochromatic radiation (1.5406 Å).

RESULTS AND DISCUSSION

Crystallization of hippuric acid

Figure 1 indicates the schematic representation of crystallization of hippuric acid crystals from buffalo urine. Length and width of square shaped crystals were measured using a scale.

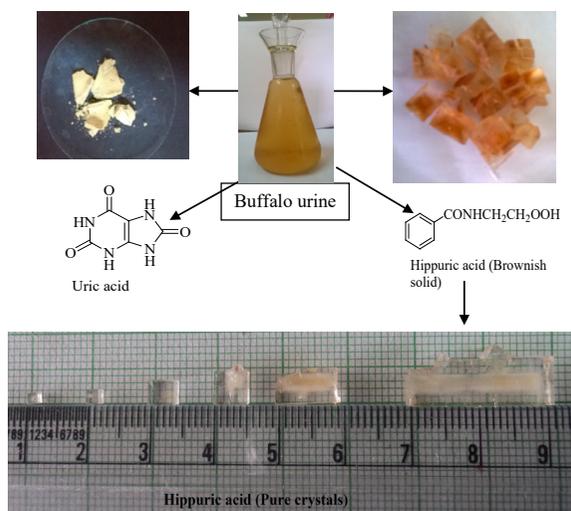


Figure 1. Schematic representation of crystallization of hippuric acid from buffalo urine.

Appearance of crystals were colourless. Minimum and maximum lengths of the crystals were 1.1 and 2.1 μm , respectively. Melting point determined was 187 to 189°C. It was freely soluble in hot water.

Characterization of hippuric acid

Figure 2 shows the UV-Vis spectrum of hippuric acid. It showed absorbance around at 200 nm. The FTIR spectrum of HA is shown in Figure 3. The intense, sharp peak at 3421 cm^{-1} in the high energy region is assigned to the NH stretching mode. The less intense peak at 3738 cm^{-1} due to OH stretching of COOH. The peak at 1595 cm^{-1} attributed to CO of COOH. The aromatic ring skeletal vibration and CH stretching, vibration are positioned at 2829 and 2719 cm^{-1} . The intense band at 1356 cm^{-1} is due to C-C=O-C vibration. There are finely resolved bands at 1114 and 744 cm^{-1} due to the aromatic ring CH and OH bending modes. The UV-Vis and IR spectral data of HA is coincided with existing literature (Vijayan *et al.*, 2005; Kumaresh and Kumar, 2014).

The powder X-ray diffraction pattern of hippuric acid is shown in Figure 4. The 2θ values were 28.21, 28.36, 28.62, 40.73, 50.48, 58.59, 58.80, 66.49, 73.95 and 87.79, respectively. The XRD pattern of the hippuric acid was compared with literature data, which matched well with the standard data. The absence of extra peaks indicates the purity of the crystals. The obtained lattice parameter values are in good agreement with the reported literature values (Vijayan *et al.*, 2005).

The DPPH scavenging activity is one of the common methods employed to determine the antioxidant activity of the plant extracts or pure compounds (Subramanian *et al.*, 2015). The HA exhibited significant DPPH radical activity. The DPPH radical scavenging activity of HA is comparable with reference compound gallic acid.

Gallic acid is well known natural antioxidant used as a food ingredient. It is used as a standard antioxidant. The concentrations of HA and gallic

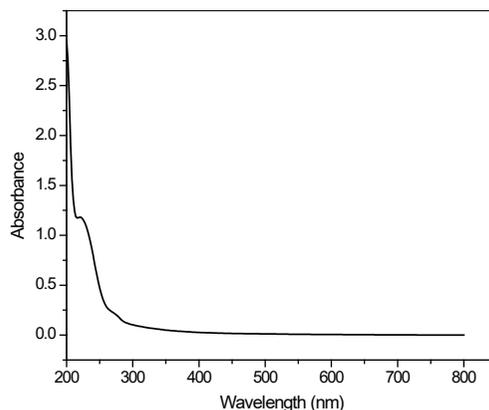


Figure 2. UV spectrum of hippuric acid.

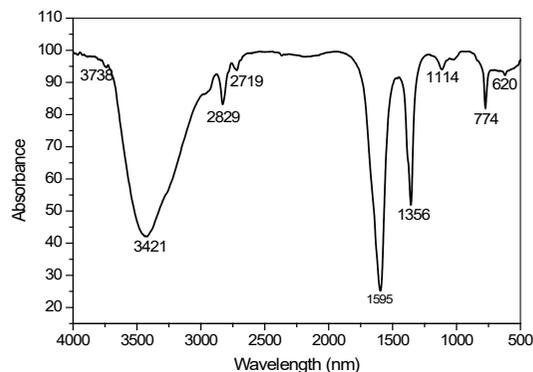


Figure 3. IR spectrum of hippuric acid.

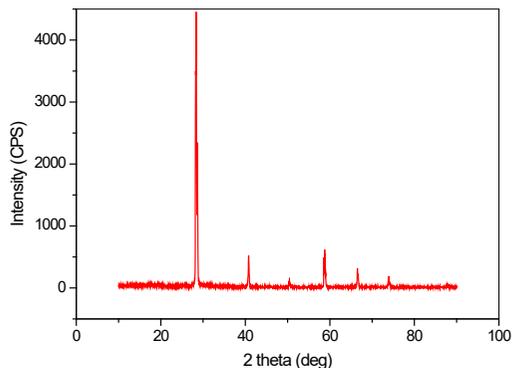


Figure 4. XRD pattern of hippuric acid.

acid used were 100, 200, 500, 800 and 1000 µg/ml. The DPPH radical scavenging activity obtained for HA was 5.66, 18.45, 26.30, 55.79 and 71.46%. Similarly, the DPPH radical scavenging activity of gallic acid obtained was 67.00, 71.61, 73.33, 74.14 and 75.45%. When at a concentration of 1000 µg/ml, the scavenging activity of HA reached 71.46%, while at the same concentration that of gallic acid was 75.45%. From the result, the DPPH radical scavenging activity is comparable with that of the gallic acid. The DPPH radical scavenging activity of HA might be due to the presence of NH and COOH groups. It indicates the antioxidant potential of hippuric acid.

Urine is composed of two major components such as uric acid and HA other than that of mineral such as potassium, nitrogen and vitamins. Primarily, uric acid was separated by distillation. Remaining brown liquid was served as a raw material for the crystallization of hippuric acid. Basically HA was found in cow urine. Later, HA has been grown by artificially with the help of chemicals such as acetone and dimethyl formamide due its nonlinear optical properties (Vijayan *et al.*, 2005; Kumaresh and Kumar, 2014).

In conclusion, HA crystals have been successfully crystallized from buffalo urine by crystallization and eco-friendly method followed by crystallization at room temperature without using any chemical. Bulk separation of the HA of urine is economically viable. The isolated hippuric acid was characterized using various techniques. The structure of the hippuric acid was confirmed by comparing the spectral data with existing literature. It possessed good DPPH radical scavenging activity which was comparable with standard antioxidant, gallic acid. Since it is freely soluble in water, it can be utilized for the development of biomaterials such nonlinear optics and nanomaterials.

Crystallization of hippuric acid from buffalo urine is consists of four steps (i) Removal of water by distillation (ii) Concentration and filtrations (iii) Autocrystallization and (iv) Recrystallization and characterization. It is concluded that the buffalo urine is a potential source of hippuric acid.

ACKNOWLEDGEMENTS

The author (R.S.) expresses his sincere thank to UGC-SERO for financial assistance by providing minor research project (MRP-5397/14 (SERO-UGC). The authors also thank to St Joeshp College and Department of Material Science and Engineering, CARE Group of Institutions, Trichy, India for providing an IR spectrum and XRD analysis.

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