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Phytochemical and Biochemical of Nagori Cow Urine: Insights into Its Antioxidant and Antibacterial Potential

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ABSTRACT: Cow urine has long been used in traditional medicine across the Indian subcontinent due to its perceived therapeutic properties. This study aimed to evaluate its phytochemical composition scientifically along with biochemical, antibacterial, and antioxidant activities. The antioxidant potential was evaluated using DPPH, hydrogen peroxide, nitric oxide, and superoxide anion scavenging assays and indicated that there was a significant dose-dependent enhancement of the free radical scavenging activity, with higher concentrations showing the highest inhibition. The inhibition of bacterial growth was demonstrated by the minimum inhibitory concentration test, which revealed the presence of antibacterial activity. The screening of phytochemicals showed that it contains phenols, tannins, flavonoids, saponins, and alkaloids. Collectively, these findings support the traditional claims and suggest that cow urine may serve as a promising natural source of therapeutic agents with significant pharmacological potential.

1. INTRODUCTION

Over centuries, both human intervention and natural selection have led to the development of livestock breeds. Cow urine has served agricultural, economic, cultural, and religious purposes in both ancient and contemporary societies. Cow holds a unique place among domestic livestock species in India, serving agricultural, economic, cultural, and religious purposes in both ancient and contemporary societies (Taberlet et al., 2008). Genetic diversity within domestic breeds enables farmers to cultivate new traits in response to changing environmental conditions, emerging diseases, and shifting market demands (McIntosh et al., 2023). It is also a component of topical moisturizers, ointments, and baths, and it can be used to make oral medications. Ancient medical sources contain numerous examples of cow urine's ability to treat a variety of human ailments. Water, urea, and a number of minerals, ions, hormones, and enzymes make up cow urine (Mahajan et al., 2020). It has potent germicidal properties due to the presence of gold hydroxide (swarnkshar), calcium, carbolic acid, creatinine, manganese, phenols, and urea. Uric acid, antioxidants, and allantoin present in cow urine have been reported to exhibit potential antioxidant activity and possible chemopreventive effects (Kumari et al., 2021). Since the Vedic era, cow urine has been used for a variety of medical purposes. Additionally, it is an essential component of Panchagavya, an Ayurvedic remedy made from five cow products: milk, curd, ghee, urine, and dung. In the past, panchagavya has been used to treat a number of diseases

such as rheumatoid arthritis, gastrointestinal problems, skin infections, and renal disorders (Bajaj et al., 2022). According to (Paliya and Deoghare 2024), cow urine is believed to support the immune system by preventing cell death, reducing the generation of free radicals, and contributes to the retardation of the aging process. The Nagori cattle breed is mostly used for transporting things. They are known for being small, strong, and able to last a long time. The name comes from the Nagaur region, and it is mostly found in the Bikaner, Jodhpur, and Nagaur districts of Rajasthan. Nagori cows are usually white and are known for their alert stance and long, narrow faces. They are regarded as one of India's most reliable draught breeds, valued especially for their speed and efficiency (Joshi et al., 2018).

This study aims to quantify key biochemical characterization, phytochemicals, antioxidant property and bioactive constituents of cow urine. The results will assess its pharmacological potential and antioxidant capacity, positioning cow urine as a promising natural source for drug discovery.

2. MATERIALS AND METHODS

2.1. Collection of cow urine

Cow urine was obtained from the well-maintained and hygienic goshala at Banasthali Vidyapith, Newai, Tonk District, Rajasthan, India (26.38-26.39° N and 75.85-75.87° E). A clinically healthy, 10-month-old female Nagori cow (heifer), maintained on a consistent diet, and was selected for sample collection. Fresh urine was collected in sterile screw-cap containers under hygienic conditions and immediately transported to the laboratory for further processing and analysis. The sample was filtered through Whatman No. 1 filter paper to remove suspended debris and precipitates. The filtered sample was transferred to an airtight sterile container and stored at 4 °C until further use.

2.2. Physicochemical properties

The physicochemical parameters of the cow urine samples, including color, taste, temperature, pH, electrical conductivity (EC), turbidity, and total dissolved solids (TDS), were evaluated immediately after sample collection. Temperature was measured using a thermometer, while pH, EC, and TDS were determined using a pH meter, EC meter, and TDS meter, respectively. Turbidity was determined using the measuring cylinder method, titration method for alkalinity (by strong acid, phenolphthalein and methyl orange indicators), and creatinine was carried out by Jaffe's turbidometric method (Gilcreas, 1966).

2.3. Qualitative analysis for phytochemical

Various qualitative tests were performed to identify the presence of biomolecules in cow urine. Molisch's test confirmed carbohydrates by the formation of a purple ring after adding α -naphthol and concentrated H_2SO_4 (Al-Wajih et al., 2022). Benedict's test indicated sugars through a color change from blue to green, yellow, orange, or brick-red upon heating. Barfoed's test showed reducing sugars by the formation of a red precipitate (Sankhalkar and Vernekar, 2016). Proteins were detected using the Biuret test, while amino acids were confirmed by the Ninhydrin test, producing a purple color after heating. Hay's sulphur test indicated bile salts based on sulfur particle settling. The sodium hypobromite test confirmed urea through observable reaction changes (Nautiyal et al., 2020). Borntrager's test revealed anthraquinone glycosides by a pink to violet color in the aqueous layer. Keller-Killani's test indicated cardiac glycosides through a blue coloration. Quinones were detected using the alcoholic KOH test by a red to blue color change (Obouayeba et al., 2015). The sample of Carr-Price reaction showed the existence of carotenoids, as it turned red after the addition of the antimony trichloride. The NaOH paper test identified coumarins with the appearance of the yellow fluorescence under ultraviolet light (Jagessar, 2017). When tannins were mixed with ferric chloride, they turned to brownish-green colour. The presence of sponins was confirmed by the formation of persistent foam and emulsion on shaking. A yellow colour was generated when the ammonia and sulphuric acid were added this shows the presence of flavonoids. A bluish-black colour formed when cow urine reacted with ferric chloride this shows the presence of phenols (Shah and Yadav, 2015).

2.4. Quantitative evaluation of phytochemical compounds

To determine the presence and concentration of the phytochemical composition of cow urine was quantitatively examined using a variety of common spectrophotometric techniques. The Folin-Ciocalteu method, which involves reacting various volumes of the sample with the reagent and measuring absorbance at 700 nm using gallic acid as a reference standard, was used to estimate the total phenolic content (Velioğlu et al., 1998). The Folin-Denis method, which uses tannic acid as the

standard and measures the formation of a blue complex at 700 nm, was also used to determine the total tannin content (Haile and Kang, 2019). The absorbance was measured at 510 nm using rutin as the standard compound, and the total flavonoid content was assessed using the aluminum chloride colorimetric method, which is based on the formation of a flavonoid-aluminum complex (Zhishen et al., 1999). The vanillin-sulfuric acid method was utilized to estimate total saponin, yielding a colored complex measured at 560 nm, with quilaia serving as the reference standard (Le et al., 2020). Additionally, absorbance was measured at 414 nm using caffeine as the standard, and total alkaloid content was ascertained by acid extraction followed by the formation of a bromothymol blue-chloroform complex (Habibian et al., 2020).

2.5. Estimation of biochemical compounds

The anthrone method, which involves dissolving 2 g of anthrone reagent in 1000 ml of sulfuric acid and reacting it with varying concentrations of cow urine, was used to determine the total carbohydrate content, the mixture was heated for five minutes before the absorbance at 510 nm was measured. Using glucose as the standard, (Nehul, 2018). The Folin-Ciocalteu (Lowry) method was used to estimate the total protein content. It involved analyzing different sample volumes and measuring absorbance at 660 nm using bovine serum albumin (BSA) as the reference standard (Lowry et al., 1951). A protein-free filtrate of cow urine was used for urea estimation. It was treated with diacetylmonoxime (DAMO) and an acid mixture before being incubated in a boiling water bath for 30 minutes. Following cooling, absorbance was measured at 480 nm using urea as the standard and distilled water as the blank (Holden, 1959). Samples were reacted with phenol reagent, sodium hydroxide, and alkaline hypochlorite, incubated for 15 minutes, and the absorbance at 600 nm was measured using ammonium sulfate as the standard to determine the ammonia content (Thompson and Watson, 1952). Using creatinine as the standard and the alkaline picrate (Jaffe's) method at 520 nm, creatinine levels were measured (Toora and Rajagopal, 2022).

2.6. Estimation of antioxidant assay

The DPPH assay was performed to determine its radical scavenging potential, with absorbance measured at 517 nm using ascorbic acid as the standard ((Sharma et al., 2019). Nitric oxide scavenging activity was assessed by incubating sodium nitroprusside in phosphate buffer with different concentrations of cow urine at 25 °C for 2.5 hours, followed by the addition of sulfanilic acid and naphthylethylenediaminedihydrochloride, and absorbance was recorded at 540 nm (Devi et al., 2017). Hydrogen peroxide scavenging activity was evaluated by mixing cow urine samples with H₂O₂ solution prepared in phosphate buffer (pH 7.4), and absorbance was measured at 230 nm using buffer as blank and ascorbic acid as the standard (Partap et al., 2014). Superoxide anion scavenging activity was determined using nitrobluetetrazolium chloride in DMSO, with absorbance recorded at 560 nm. These assays collectively indicate the antioxidant potential of cow urine (Lalhminghlui and Jagetia, 2018).

2.7. Antibacterial activity of cow urine

The antibacterial activity of cow urine was evaluated using the disk diffusion method under in vitro conditions against *Escherichia coli* (a Gram-negative strain) and *Bacillus subtilis* (a Gram-positive bacterium). Sterile 5 mm diameter disks were rinsed in cow urine dilutions at different concentrations 5 µl, 10 µl, and 15 µl (Sathasivam et al., 2010; Nishanth et al., 2010). Chloramphenicol (30 µg) was used as the standard control. The results demonstrated a concentration-dependent antibacterial activity (Randhawa and Sharma, 2015).

2.8. Statistical analysis

All assays were performed in triplicates. Data were presented as mean $\pm$ standard deviation (SD) of three determinations. The inhibitory concentration (IC₅₀) was calculated by nonlinear regression analysis (GraphPad prism version 8.0, GraphPad Software, USA).

3. RESULTS

3.1. Physicochemical Properties

Fresh cow urine from the Nagori breed was pale yellow in color with a salty, bitter, and slightly pungent taste. It had a temperature of 33.5°C, pH of 8.3, conductivity of 4.20 mS/cm, turbidity of 0.31, TDS of 2.11 mg/l, alkalinity of 960.2 mg/l, and creatinine content of 1.15 mg/l.

3.2. Qualitative analysis for phytochemical of cow urine

Table 1 shows the phytochemical analysis of cow urine to indicate the presence carbohydrates, sugars, reducing sugars, proteins, amino acids, bile salts, urea, cardiac glycosides, quinones, carotenoids, coumarins, tannins, saponins, flavonoids, and phenols.

Table 1: Qualitative tests for phytochemical of the fresh cow urine sample “+” indicates a positive reaction (presence detected), whereas “-” indicates a negative reaction (not detected) under the test conditions.

S. No.	Phytochemical	Qualitative tests	Sample
1	Carbohydrates	Molisch's test	+
2	Sugars	Benedict's test	+
3	Reducing sugars	Barfoed's test	+
4	Proteins	Biuret test	+
5	Amino acids	Ninhydrin test	+
6	Bile salts	Hay's sulphur test	-
7	Urea	Sodium Hypobromite test	+
8	Anthraquinone glycosides	Bontrager's test	-
9	Cardiac glycosides	Keller-Kilani's test	+
10	Quinones	Alcoholic KOH test	+
11	Carotenoids	Carr-Price reaction test	+
12	Coumarins	NaOH paper test	+
13	Tannins	Ferric chloride test	+
14	Saponins	Frothing test	+
15	Flavonoids	Ammonia test	+
16	Phenols	Ferric chloride test	+

3.3. Quantitative evaluation of phytochemical compounds in cow urine

3.3.1. Total phenolic content (TPC)

The total phenolic content (TPC) was determined using a calibration curve generated from various concentrations of gallic acid ($y = 0.3714x + 0.3597$, $R^2 = 0.9524$) and expressed as gallic acid (GAE) equivalent. TPC in cow urine was measured at different volumes (200, 400, 600, 800, and 1000 μ l). The highest TPC concentration, 10.46 ± 0.39 mg GAE/ml, was observed at 1000 μ l, while the lowest, 2.16 ± 0.21 mg GAE/ml, was recorded at 200 μ l is illustrated in figure 1(a).

3.3.2. Total tannin content (TTC)

The total tannin content (TTC) was determined using a calibration curve generated from various concentrations of tannic acid ($y = 2.5064x + 0.1263$, $R^2 = 0.991$) and expressed as tannic acid (TA) equivalent, TTC in cow urine was measured at different volumes (200, 400, 600, 800, and 1000 μ l). The highest TTC concentration 2.59 ± 0.06 mg TA/ml, was observed at 1000 μ l, while the lowest, 0.25 ± 0.09 mg TA/ml, was recorded at 200 μ l as illustrated in figure 1(b).

3.3.3. Total flavonoid content

The total flavonoid content (TFC) in cow urine was determined using a calibration curve prepared from gallic acid ($y = 0.7689x + 0.0789$, $R^2 = 0.9669$) and expressed as rutin (RUT) equivalent. TFC was measured at different volumes (200, 400,

600, 800, and 1000 μ l). The highest flavonoid concentration (4.45 ± 0.08 mg RUT/ml) was observed at 1000 μ l, while the lowest (0.44 ± 0.08 mg RUT/ml) was recorded at 200 μ l, as shown in figure 1(c).

3.3.4. Total saponin content

The total saponin content (TSC) was determined using a calibration curve derived from different concentrations of quillaja (QJ) ($y = 0.7086x + 0.0347$, $R^2 = 0.9904$) and is expressed as quillaja (QJ) equivalent. TSC in cow urine was determined at various volumes (200, 400, 600, 800, and 1000 μ l). The maximum TSC concentration, 8.95 ± 0.26 mg QJ/ml, was recorded at 1000 μ l, whereas the minimum, 1.96 ± 0.29 mg QJ/ml, was documented at 200 μ l in figure 1(d) illustrates this.

3.3.5. Total alkaloid content

The total alkaloid content (TAC) was determined using a calibration curve derived from different concentrations of caffeine ($y = 0.2x + 0.01437$, $R^2 = 0.9841$) and is expressed as caffeine (Cf) equivalent. TAC in cow urine was determined at various volumes (200, 400, 600, 800, and 1000 μ l). The maximum TAC concentration, 12.72 ± 0.35 mg Cf/ml, was recorded at 1000 μ l, whereas the minimum, 2.12 ± 0.30 mg Cf/ml, was documented at 200 μ l in figure 1(e) illustrates this.

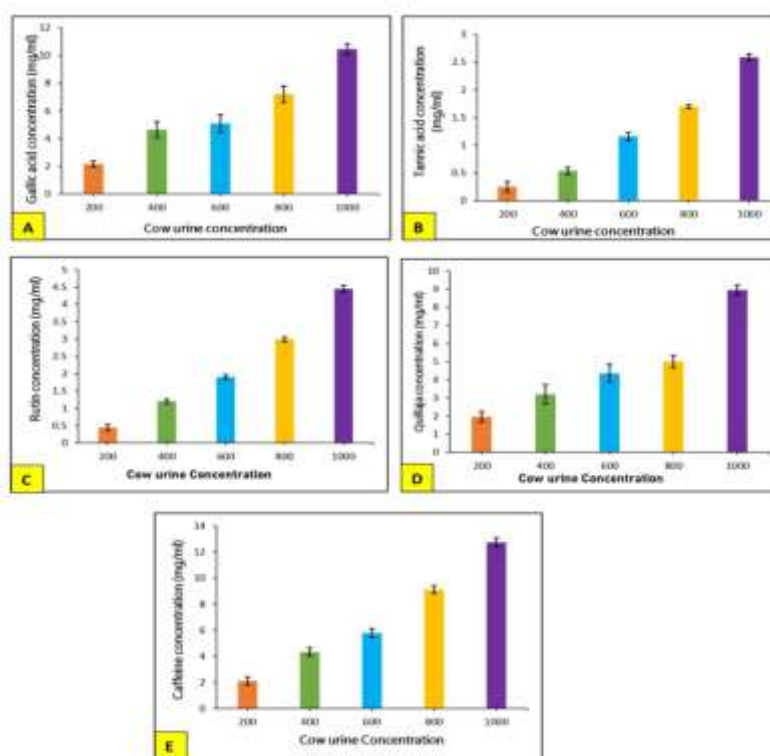


Figure 1: Quantitative analysis of phytochemical compounds in cow urine: (a) total phenolic content (b) total tannin content (c) total flavonoid content (d) total saponin content (e) total alkaloid content

3.4. Quantitative evaluation of biochemical compounds in cow urine

3.4.1. Estimation of total carbohydrates

The total carbohydrates content was estimated in different concentration of cow urine (100, 200, 300, 400, and 500). Total estimation of carbohydrates in cow urine by using the regression equation of the standard curve ($y = 0.0007x + 0.0235$, $R^2=0.9946$). The maximum concentration of carbohydrates was found in 500 μ l of cow urine (1.06 ± 0.06 mg/ml). The minimum concentration was observed in 100 μ l of cow urine (0.20 ± 0.05 mg/ml). The percentage of total carbohydrates increased with the increasing volume of cow urine, as shown in figure 2(a).

3.4.2. Estimation of total protein

The protein content was estimated at varying concentrations of cow urine (200, 400, 600, 800, and 1000 μL) using the regression equation derived from the standard curve ($y = 0.3489x + 0.0079$, $R^2 = 0.9958$). The highest protein concentration (1.34 ± 0.06 mg/ml) was observed in 1000 μL of cow urine, while the lowest (0.37 ± 0.06 mg/ml) was recorded in 200 μL . The results indicate a progressive increase in total protein content with increasing cow urine volume, as shown in figure 2(b).

3.4.3. Estimation of urea

The urea content was quantified at varying volumes of cow urine (200, 400, 600, 800, and 1000 μL) using the regression equation derived from the standard curve ($y = 1.114x + 0.0006$, $R^2 = 0.9801$). The highest urea concentration (2.11 ± 0.06 $\mu\text{g}/\text{mL}$) was observed in 1000 μL of cow urine, whereas the lowest concentration (0.74 ± 0.06 $\mu\text{g}/\text{mL}$) was recorded in 200 μL . The results demonstrate a progressive increase in total urea content with increasing cow urine volume, as illustrated in figure 2(c).

3.4.4. Estimation of ammonia

Total ammonia content in cow urine was quantified at varying concentrations (100, 200, 300, 400, and 500 μL) using a standard curve regression equation ($y = 0.0021x + 0.0343$, $R^2 = 0.9946$). The highest ammonia concentration (1.47 ± 0.04 $\mu\text{g}/\text{mL}$) was observed at 500 μL , while the lowest (0.29 ± 0.019 $\mu\text{g}/\text{mL}$) was recorded at 100 μL as shown in figure 2(d), ammonia levels increased progressively with rising sample volume, indicating a concentration-dependent pattern.

3.4.5. Estimation of creatinine

Creatinine content in cow urine was estimated at varying concentrations (100, 200, 300, 400, and 500 μL) using the regression equation derived from the standard curve ($y = 0.9714x + 0.1203$, $R^2 = 0.9946$). The highest creatinine concentration, 1.23 ± 0.04 mg/mL, was observed at 500 μL , while the lowest concentration, 0.43 ± 0.01 mg/mL, was recorded at 100 μL is shown in figure 2(e).

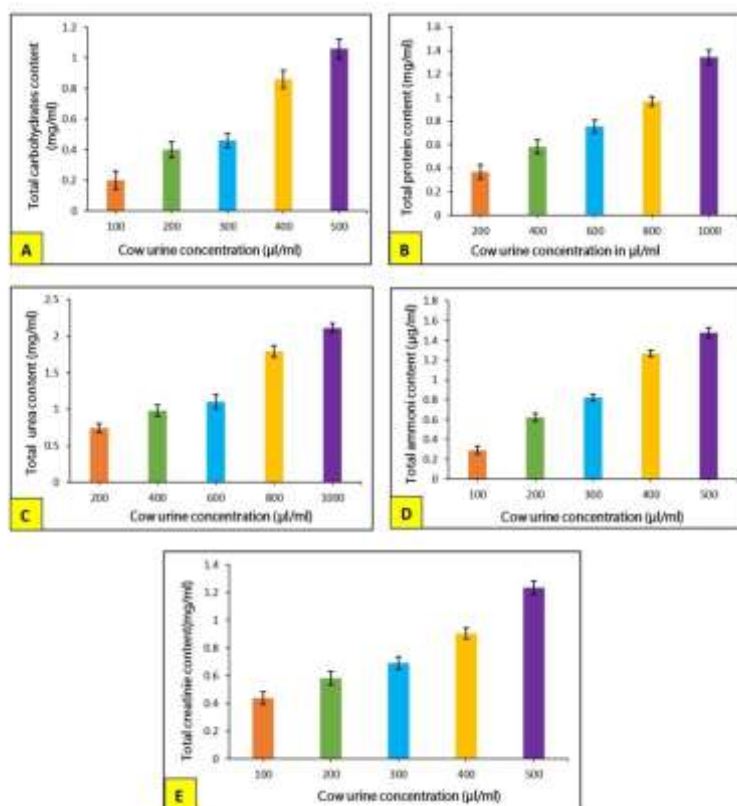


Figure 2: Quantitative evaluation of biochemical compounds in cow urine: (a) total content of carbohydrates (b) total content of protein (c) total content of urea (d) total content of ammonia (e) total content of creatinine

3.5. Evaluation of antioxidant activity in cow urine

3.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The antioxidant capacity of cow urine was evaluated using the DPPH free radical scavenging assay at concentrations ranging from 120 to 200 $\mu\text{l/ml}$. Maximum inhibition (93.88%) was observed at 200 $\mu\text{l/ml}$, as shown in figure 3(a) the lowest (81.15%) occurred at 120 $\mu\text{l/ml}$, with an IC_{50} value of 67.16 $\mu\text{l/ml}$, indicating strong antioxidant potential. Ascorbic acid, used as a standard, showed 84.21% inhibition at 200 $\mu\text{g/ml}$ and an IC_{50} of 78.10 $\mu\text{g/ml}$.

3.5.2. Nitric oxide (NO) Scavenging Assay

The nitric oxide scavenging method was used to evaluate the antioxidant potential of cow urine. Absorbance readings were taken for each tested concentration (120, 140, 160, 180, and 200 $\mu\text{l/ml}$). The IC_{50} values calculated for ascorbic acid (AA), used as the standard, and for the cow urine samples reflect their respective antioxidant efficiencies. Among the tested concentrations, the highest nitric oxide radical inhibition was recorded at 200 $\mu\text{l/ml}$ of cow urine, showing an inhibition rate of 94.79%. As shown in figure 3(b) the least inhibition was observed at 75.57% at 120 $\mu\text{l/ml}$ of cow urine. The IC_{50} for cow urine was 87.22 $\mu\text{l/ml}$. Therefore, we found that both the lower and higher concentrations of cow urine effectively neutralized the free radicals generated in the reaction mixture. Ascorbic acid was taken as a standard, which showed the maximum nitric oxide radical inhibition (97.28 %) at 200 $\mu\text{g/ml}$, while the lowest inhibition (82.31 %) was observed at 120 $\mu\text{g/ml}$. The IC_{50} value of ascorbic acid is 90.12 $\mu\text{g/ml}$.

3.5.3. Hydrogen peroxide (H_2O_2) radical scavenging activity analysis

Cow urine's antioxidant activity was assessed using a hydrogen peroxide radical scavenging analysis. Measurements of absorbance were made for all tested concentrations (120, 140, 160, 180, and 200 $\mu\text{l/ml}$). The IC_{50} values for both the reference antioxidant, ascorbic acid (AA), and the cow urine samples indicate their respective capacities to neutralize hydrogen peroxide. The maximum inhibition % of hydrogen peroxide radical was observed at 98.36% at 200 $\mu\text{l/ml}$ of cow urine. As shown in figure 3(c) the least inhibition was observed at 85.92% at 120 $\mu\text{l/ml}$ of cow urine. The IC_{50} for cow urine was 78.50 $\mu\text{l/ml}$. Therefore, we found that both the lower and higher concentrations of cow urine effectively neutralized the free radicals generated in the reaction mixture. Ascorbic acid was taken as a standard, which showed the maximum hydroxide peroxide radical inhibition (91.24%) at 200 $\mu\text{g/ml}$, while the lowest inhibition (88.64 %) was observed at 120 $\mu\text{g/ml}$. The IC_{50} value of ascorbic acid is 82.25 $\mu\text{g/ml}$.

3.5.4. Superoxide anion scavenging assay

The antioxidant capacity of cow urine was evaluated using the superoxide anion scavenging assay at concentrations ranging from 120 to 200 $\mu\text{l/ml}$. A dose-dependent increase in activity was observed, with maximum inhibition of 91.59% at 200 $\mu\text{l/ml}$ and minimum inhibition of 71.05% at 120 $\mu\text{l/ml}$. The IC_{50} value for cow urine was calculated as 87.75 $\mu\text{l/ml}$, indicating strong free radical scavenging potential. Ascorbic acid, used as the standard antioxidant, showed maximum inhibition of 94.46% at 200 $\mu\text{g/ml}$ and minimum inhibition of 79.34% at 120 $\mu\text{g/ml}$, with an IC_{50} value of 91.16 $\mu\text{g/ml}$ (figure 3d).

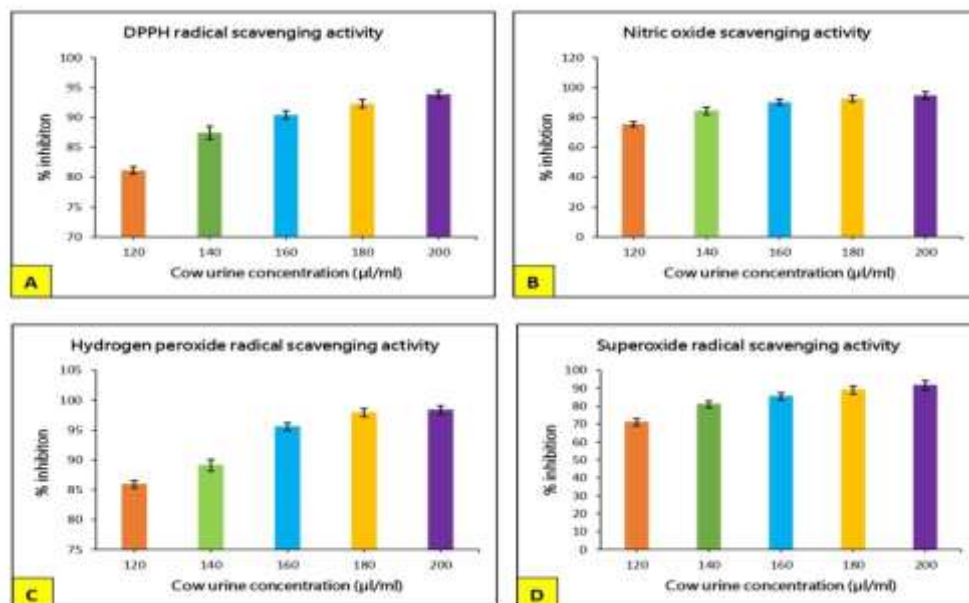


Figure 3: Evaluation of antioxidant activity in cow urine: (a) DPPH scavenging assay (b) Nitric oxide scavenging assay (c) Hydrogen peroxide scavenging assay (d) Superoxide anion scavenging assay

3.6. Antibacterial Activity of Cow Urine

The disk diffusion method was used to assess the antibacterial activity of cow urine against *Bacillus subtilis* and *Escherichia coli*. At 5 μl, *E. coli* showed a minimal inhibition zone of 1 mm, which grew to 3 mm and 5.5 mm at 10 μl and 15 μl, respectively. *B. subtilis*, on the other hand, demonstrated a higher level of inhibition with inhibition zones of 3.5 mm, 6 mm, and 13 mm at 5 μl, 10 μl, and 15 μl, respectively (figure 4).

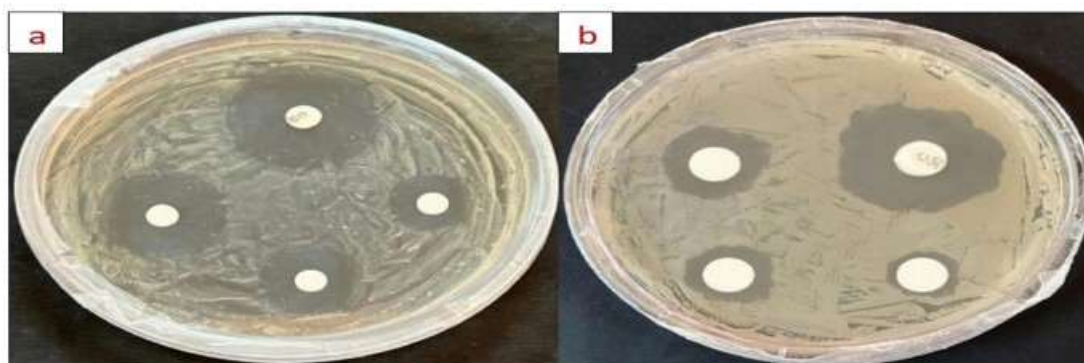


Figure 4: (a) Antimicrobial activity of cow urine on *Bacillus subtilis*, (b) Antimicrobial activity of cow urine on *Escherichia coli*

4. Discussion

The present study provides an integrated characterization of fresh Nagori cow urine using physicochemical, biochemical, phytochemical, antioxidant, antibacterial, GC-MS, and FT-IR approaches. The findings demonstrate that the investigated sample contains a diverse mixture of biochemical and phytochemical constituents and exhibits measurable antioxidant and antibacterial activities under in-vitro conditions. These observations are broadly consistent with previous investigations of cow urine, although the composition and biological activity of urine can vary according to breed, age, physiological condition, diet, geographical location, environmental factors, and sample-processing procedures. Recent metabolomic research involving multiple cattle breeds has further emphasized the chemical diversity of cow urine and demonstrated that breed and animal-related variables can influence its metabolite profile (Kushwaha et al., 2025).

The fresh Nagori cow urine examined in the present investigation exhibited an alkaline pH together with measurable electrical conductivity, turbidity, total dissolved solids, alkalinity, and creatinine. The alkaline character of cow urine is consistent with its complex mixture of nitrogenous metabolites, electrolytes, minerals, and organic compounds. Earlier investigations have demonstrated that cow urine contains urea, creatinine, uric acid, minerals, enzymes, hormones, and other organic constituents (Mahajan et al., 2020; Nautiyal and Dubey, 2021). The physicochemical characteristics observed in the present study therefore support the description of cow urine as a chemically complex biological fluid rather than a simple aqueous waste product.

The biochemical screening in the present study demonstrated the presence of carbohydrates, proteins, amino acids, urea, ammonia, and creatinine. These findings are consistent with earlier studies investigating the biochemical composition of cow urine. Sharma et al. (2019) evaluated cow urine for biochemical, antioxidant, and antibacterial properties and reported the presence of amino acids together with measurable antioxidant and antimicrobial activities.

The occurrence of proteins and amino acids is particularly relevant because nitrogen-containing biomolecules may contribute to the overall biological complexity of cow urine. However, the present biochemical assays do not establish the identity or biological function of individual proteins or peptides. Therefore, the detected biochemical constituents should be considered components of the complex urine matrix rather than individually responsible for the biological effects observed in the present study. Qualitative phytochemical screening demonstrated the presence of carbohydrates, sugars, reducing sugars, proteins, amino acids, bile salts, urea, cardiac glycosides, quinones, carotenoids, coumarins, tannins, saponins, flavonoids, and phenols. The detection of several chemically distinct phytochemical classes suggests that the biological activity of cow urine is likely associated with a complex mixture of compounds rather than a single active molecule. The quantitative estimation further demonstrated the presence of phenolic, tannin, and flavonoid constituents. The total phenolic content increased with increasing sample concentration, supporting the presence of phenolic substances within the investigated Nagori cow urine. Phenolic compounds are of particular interest because their hydroxyl groups can participate in hydrogen- and electron-transfer reactions and may contribute to radical-scavenging activity. The present observation agrees with previous research reporting phenolic constituents in cow urine and cow urine-derived preparations. Manjramkar et al. (2019) reported measurable total phenolic content in cow urine distillate and suggested a relationship between phenolic constituents and antioxidant potential. The present results therefore provide additional evidence that phenolic constituents may contribute to the antioxidant properties of cow urine, although direct causal relationships cannot be established from total phenolic measurements alone. Similarly, flavonoids were detected in the present sample and increased with increasing sample concentration. Flavonoids are widely recognized for their capacity to interact with reactive oxygen species through hydrogen donation, electron transfer, and metal-chelating mechanisms. Nevertheless, the total flavonoid assay used in this study provides an estimate of flavonoid-equivalent content and does not identify individual flavonoid molecules. Therefore, further LC-MS/MS-based characterization would be required to determine the specific flavonoids present. The detection of tannins and saponins is also noteworthy because these compounds have previously been associated with antimicrobial and antioxidant properties. The presence of these compounds may therefore contribute to the multifunctional biological profile observed in the present study. However, variation in breed, diet, physiology, and sample processing may account for differences between the present Nagori sample and cow urine examined in previous studies.

The concentration-dependent antioxidant activity observed in the present study represents one of the major findings. Nagori cow urine demonstrated radical-scavenging activity against DPPH, nitric oxide, hydrogen peroxide, and superoxide anion. The DPPH findings are consistent with Sharma et al. (2019), who reported antioxidant activity of cow urine using a DPPH assay and demonstrated a concentration-dependent response. Similarly, Joshi et al. (2019) reported antioxidant activity in cow urine samples obtained from different climatic and altitudinal regions of Nepal, suggesting that environmental and geographical conditions may contribute to variation in antioxidant capacity.

The use of several antioxidant assays in the present investigation provides broader chemical evidence because DPPH, nitric oxide, hydrogen peroxide, and superoxide assays involve different reactive species and reaction mechanisms. The increasing activity observed with increasing sample concentration across these assays suggests that several constituents may contribute simultaneously to radical scavenging. Phenolic and flavonoid compounds are plausible contributors, although the present experimental design cannot establish a direct causal relationship between the measured phytochemical classes and antioxidant activity.

Recent research also supports continued investigation of the antioxidant potential of cow urine preparations. A study on cow urine distillate-derived copper nanoparticles reported that cow urine distillate possesses antioxidant and antimicrobial characteristics and can act as a biological reducing medium during nanoparticle synthesis (Angle et al., 2025). Although that investigation examined cow urine distillate-mediated nanoparticle synthesis rather than fresh Nagori urine, it provides contemporary evidence that constituents present in cow urine preparations can participate in redox-related biological and chemical processes.

The antioxidant findings should nevertheless be interpreted within the limitations of chemical in-vitro assays. Radical-scavenging activity measured in a reaction mixture does not directly demonstrate antioxidant efficacy in an organism. Bioavailability, metabolism, absorption, tissue distribution, and achievable physiological concentrations may substantially influence biological effects. Consequently, the present results demonstrate chemical antioxidant potential rather than established therapeutic antioxidant efficacy.

The present study also demonstrated antibacterial activity against both *Escherichia coli* and *Bacillus subtilis*, with inhibition increasing at higher tested sample concentrations. *B. subtilis* showed greater inhibition than *E. coli* under the experimental conditions used. Differences in bacterial susceptibility may reflect variations in cell-envelope architecture, membrane permeability, efflux systems, metabolic characteristics, and interactions with individual constituents of cow urine. These findings are consistent with the earlier study of Sharma et al. (2019), which reported antibacterial activity of cow urine together with antioxidant activity. Nautiyal and Dubey (2021) further demonstrated antibacterial activity in bioactive fractions of Badri cow urine against several bacterial pathogens. Their work is particularly relevant because it combined antibacterial testing with FT-IR and GC-MS characterization, providing evidence that the chemical composition of cow urine fractions may be associated with their biological activity. The antibacterial properties of cow urine have also been investigated in more recent studies. Pant et al. (2024) reported antibacterial activity of cow urine distillate against five bacterial strains, with activity varying among organisms and concentrations. Their study also used in-vitro and in-silico approaches to investigate possible mechanisms and identified ferulic acid and 2-hydroxycinnamic acid as compounds showing interactions with bacterial DNA gyrase. Although these findings were obtained using cow urine distillate rather than fresh Nagori cow urine, they provide a possible mechanistic framework for investigating antibacterial constituents in future studies. Breed-specific investigations provide additional support for the importance of considering animal origin. Paliya and Deoghare (2025) examined urine from Red Sindhi cattle and reported antibacterial and cytoprotective properties in vitro. Their findings included activity against Gram-positive and Gram-negative bacteria and demonstrate that breed-specific characterization may be useful when evaluating the biological properties of cow urine. Together, these recent studies reinforce the importance of evaluating cow urine from individual breeds rather than assuming that results obtained from one breed can be directly generalized to all cattle. Microbiological quality is another important consideration when interpreting antibacterial activity of fresh urine. Rathore et al. (2023) examined the bacteriological quality of fresh and distilled Badri cow urine and reported the absence of several tested pathogens, although measurable aerobic bacterial counts were detected. This finding emphasizes the importance of aseptic collection, rapid processing, controlled storage, and microbiological quality assessment when studying fresh cow urine. Such controls are particularly relevant because contamination during collection or storage could potentially influence antibacterial assays and confound interpretation of the intrinsic activity of urine constituents.

Overall, the present investigation provides a comprehensive physicochemical, biochemical, phytochemical, antioxidant, and antibacterial characterization of fresh Nagori cow urine. The agreement of the findings with previous studies on cow urine antioxidant, antibacterial, and phytochemical characteristics supports the view that cow urine contains diverse constituents associated with measurable in-vitro bioactivity. However, these findings should be regarded as evidence of biological potential rather than proof of therapeutic efficacy. Further studies involving targeted chemical characterization, isolation of individual constituents, mechanistic investigations, and appropriate in-vivo models are required to establish the specific compounds and mechanisms responsible for the observed activities.

5. CONCLUSION

The present study provides an integrated physicochemical, biochemical, phytochemical, antioxidant, antibacterial, GC-MS, and FT-IR characterization of fresh urine obtained from a Nagori cow. The findings demonstrated that the sample contained several biochemical constituents, including carbohydrates, proteins, urea, ammonia, and creatinine, together with diverse phytochemical classes such as phenols, flavonoids, tannins, saponins, and alkaloids. The observed antioxidant activity across

DPPH, nitric oxide, hydrogen peroxide, and superoxide anion scavenging assays indicates that Nagori cow urine possesses measurable in-vitro free-radical-scavenging capacity. Furthermore, antibacterial activity was observed against both *Bacillus subtilis* and *Escherichia coli*, with greater inhibition at higher tested concentrations. GC-MS analysis revealed a chemically diverse profile comprising organic acids, phenolic-related compounds, amino-acid-associated metabolites, heterocyclic compounds, and fatty-acid amides, while FT-IR analysis confirmed the presence of several characteristic functional groups. Collectively, these findings demonstrate the chemical complexity and measurable biological activities of Nagori cow urine under the experimental conditions investigated. However, the study involved a sample obtained from a single animal, and the in-vitro assays do not establish therapeutic efficacy. Further investigations involving multiple animals, standardized sampling, targeted metabolomic analysis, compound isolation, mechanistic cellular studies, and controlled in-vivo experiments are required to identify the constituents responsible for the observed activities and determine their biological significance.

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Author contributions

Anjali Sachan: Conceptualization, Investigation, Methodology, Data Curation, Formal Analysis, Writing-Original Draft.

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Conflict of interest

The authors declare that there is no conflict of interest.

Ethics Statement

Ethical approval was not required for this study.

Data availability statement

The data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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