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Research Article

POTENTIAL SOURCE OF FRESH AND PHOTOACTIVATED GOMUTRA FOR STUDY OF ANTIOXIDANT AND ANTIPATHOGENIC ACTIVITIES AGAINST VARIOUS PATHOGENS

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ABSTRACT

Nowadays, the increase in use of antimicrobial drugs has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents and ongoing epidemics of HIV infection. These drugs are economically too expensive and inadequate for the treatment of diseases in developing countries, and it also has some serious side effects. Some pathogens have also become resistant to many commercially used antibiotics. Therefore, there is a need to develop new infection-fighting strategies from natural source to control pathogenic infections without cause any side effects. The cow urine has a best source of natural compounds with antimicrobial and antioxidant properties. In this present study, the cow urine was collected at different time intervals and the antibacterial activity of Photo activated, distillate and fresh raw cow urine were tested by agar well and pour plate method using the pathogenic microbes like *pseudomonas auerogenisa, Bacillus subtilis, and Klebsiella pneumonia*. The antioxidant activity of the Cow Urine and its fractions has been evaluated by Free Radical Scavenging Activity using DPPH assay method and Reducing Power Assay. In this result we found that the fresh and photo activated cow urine evidences for better antioxidant and antimicrobial activity by inhibiting *Klebsiella pneumonia* (31 and 27mm) compared to distillate. We conclude that the fresh and photo activated gomutra could be a potential source of natural antioxidant that would have greater importance as supportive therapy in slowing oxidative stress related degenerative diseases and also act as effective tool for inhibiting pathogenic infections.

Keywords: DPPH, Reducing Power, Photo activated and Distillate.

Among Indians, cow is considered to be a spiritual animal and they worshiped them. Every Products obtained from the cow was useful to the mankind such as dung, urine, milk and milk products. People believed that they are rich in medicinal property, among them the cow urine plays a major role [1]. It is enriched in medicinal values and also used as a power full disinfectant. Cow urine has potent pharmacological importance as its medicinal utility has been mentioned already in Ayurveda. Cow urine contains many essential components such as minerals (N, P, K, Ca,Cl) proteins and etc. Cow urine has been identified to be effective against, certain cardiac and kidney diseases, indigestion, stomach ache, edema, skin disease, epilepsy, anemia, constipation, respiratory disease etc [2]. It is also used by traditional homoeopaths in combination with herbs for treatment of fever, epilepsy and anemia. Cow urine exhibits both antioxidant and antimicrobial activities against certain drug resistant bacterial and fungal strains [3,4].

Antimicrobial agents play a key role in control of pathogenic diseases. The increase in use of antimicrobial drugs has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection [5]. These drugs are economically too expensive and inadequate for the treatment of diseases in developing countries, and it also has some serious side effects. Some pathogens have also become resistant to many commercially used antibiotics. Therefore, there is a need to develop new infection-fighting strategies to control microbial infections [6]

Free radicals are groups of atoms with an unpaired number of electrons and can be formed when oxygen interacts with certain molecules. These are generated by air pollution, smoking and alcohol. Not only by the external factors, our daily food and water contains the free radicals. The free radicals in our body damage the cell and its cellular components thus its leads to many disorders and cause genetic disease. Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged and used to maintain the level of free radicals and oxygen [**7**].

Cow urine could effectively inhibit the microbial infections and has great aesthetic and medicinal value though its utility has been mentioned in holy texts of Indian literature. Cow urine has certain volatile and nonvolatile components, which might have very high antimicrobial activity. After photo activation and purification cow urine became free from microbes and has massive toxic potential to kill drug resistant bacterial strains. It has been observed that important forest dwelling cows secrete so many herbal compounds in urine. In such cows plant origin dietary organic and inorganic compounds effectively get absorbed in the rumen and digested by bacterial activity. But there are some compounds, which do not disturb by any microbial enzyme action and secreted in their natural form in cow urine [4].

In this present study, we have studied antioxidant and antibacterial activity of Photo activated, distillate and fresh raw cow urine for mentioned pathogens.

MATERIALS AND METHODS

Bacterial Strains Collection

The Bacterial strains were collected includes *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* strain.

Strains Maintenance

The collected strains were inoculated into Nutrient broth, IndoleTryptone broth, MR-VP broth and Nutrient agar. The cultivated strains were kept in orbital shaker (Remi Laboratories) at 37º C temperature for further studies.

Collection of Gomutra

Gomutra (Cow urine) was collected from disease free cow on different time intervals (Morning, Afternoon and Night) in sterile container and stored at refrigerator (4º C) for further analysis.

Preparation of Gomutra Fractions

The cow urine was fractionated into different fractions such as natural raw, distillate and photo activated cow urine. In natural raw cow urine, the urine was directly collected in sterile container from the cow. For preparation of cow urine distillate, the urine was distilled at 100°c using distillation apparatus. The single distilled cow urine was acidified by lowering the pH below 2.0 with the addition of 85% orthophosphoric acid. The cow urine was again distilled at 100° c using the distillation apparatus to remove ammonia. Whereas in photo activated cow urine, the urine was kept in sunlight for 48 hours in a transparent sterile glass bottle. Then the urine was filtered through whattman No.1 filter paper to remove it from debris and precipitated materials. The prepared fractions were stored in refrigerator at 4° c for the further usage.

Antioxidant Assay

There are two different invitro methods were used to determine the antioxidant activity of cow urine and its fraction namely the DPPH (1, 1 diphenyl-2-picrylhydrazyl) free radical scavenging assay and reducing power. These assays were also supported by determination of phenolic contents in cow urine and from all the assays the average value was considered.

DPPH radical scavenging assay

The free radical scavenging capacity of the cow urine was determined using DPPH assay. Methanolic solution of test samples and ascorbic acid (standard) were prepared at various concentrations (100,200,400,800and1000 µg/ml or µl/ml). To a set of test tubes, 2.9 ml of DPPH solution (100µg/ml in methanol) and 0.1 ml of varying concentrations of test samples and ascorbic acid were added. The mixture was then shaken vigorously and allowed to stand in dark for 30 min. Absorbance was measured at 517nm using a spectrophotometer. A control was prepared by using 0.1 ml of methanol and 2.9 ml of DPPH radical solution. Percentage scavenging of DPPH radical was calculated by comparing the absorbance between the sample and control [5].

% scavenging of DPPH radical = $[(A_{blank}-A_{sample})/A_{blank}]\times100$

Where, A_{blank} and A_{sample} are the absorbance values of the control (blank) and the sample.

Reducing Power Assay

The reducing power of the cow urine was determined by using ferricyanide. Various concentrations of 100-1000 µg cow urine in 0.1 mL of deionized water were mixed with 2.5 mL of 0.2M, pH 6.6 phosphate buffer and 2.5 mL potassium ferricyanide [$K_3Fe(CN_6)$]. The mixture was incubated at 50ºC for 20 mins. Aliquots of 2.5 mL, 10 % tricholoroacettic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. 2.5ml of upper layer solution was mixed with 2.5 mL of distilled water and 0.5ml, 1% $FeCl_3$ freshly prepared solution was added. The absorbance was measured at 700 nm. As a control, ascorbic acid was used.

Determination of total phenolic compounds

Total phenolic content was determined by the Folin-Ciocalteau method. Weighed accurately 100 mg of standard Gallic acid and dissolved in 100ml distilled water. Various concentrations of gallic acid (10-100 µg/ml) were prepared. From these concentrations 1mL was mixed with 5 ml Folin- Ciocalteau reagent (diluted tenfold) and then 4ml with (7.5%) sodium carbonate. The absorption was read at 765 nm after 30 min at 20°C and the calibration curve was drawn. One ml of cow urine (1mg/ml) was mixed with the same reagents as described above and after 30 min the absorption was measured at 765 nm for the determination of phenolic contents). Total phenolic contents of the samples were quantified by calibration curve obtained from measuring the known concentrations of gallic acid and are expressed as Gallic acid equivalent (GAE).

Antimicrobial Activity of Gomutra

(i)Well Diffusion Method

The antibacterial activity of cow urine against various bacterial strains *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiellapneumoniae*was performed by agar well diffusion method. Nutrient agar medium plates were prepared, sterilized and 0.5 mL of different bacterial cultures was inoculated in these plates. After semi solidification, wells were made uniformly at 10 mm size and different fractionated samples were poured into each well on all plates using 10-50 µL micropipettes (5, 10, 25 and 30 µL). The plates were incubated at 37°C for 48 hours. The results of inhibition were evaluated by measuring diameter of the zone of inhibition surrounding the discs.

(ii)Pour Plate Method

1ml of fractionated cow urine sample was poured in to sterilized petridishes containing nutrient agar. The spores of *Bacillus subtilis, Pseudomonas aeruginosa*and*Klebsiella pneumonia* were spread in the plate and kept incubation for 48 hours to observe the inhibition of bacterial growth pattern.

(iii) Comparison of Antibiotics and gomutra

For this analysis, antibiotics and cow urine were subjected to susceptibility test. The diameter of the zone of inhibition around the discs was compared to the standard antibiotics tetracycline.

Phytochemical Screening for gomutra

The cow urine was analyzed to preliminary phytochemical screening for carbohydrates, glycosides, phenol, proteins and amino acids.

Table 1: DPPH radical scavenging activity - Fresh and Photo activated cow urine.

S.NO	SAMPLES (µg/mL)	%SCAVENGING OF DPPH at 517nm					
		100	200	400	600	800	1000
1.	Fresh cow urine	18.5	31.5	49.4	56.8	60.5	66.4
2.	Photo activated cow urine	16.4	24.6	33.8	48.9	51.9	53.9
3.	Ascorbic Acid	33.3	42.6	58.6	68.3	79.0	89.6

Table 2: Reducing power Assay- Fresh and photo activated cow urine.

S.NO	SAMPLES (µl/mL)	Absorbance at 700nm					
		100	200	400	600	800	1000
1.	Cow URINE	0.413	0.578	0.688	0.847	0.957	1.032
2.	Photo activated Cow URINE	0.358	0.487	0.546	0.721	0.899	0.987
3.	ASCORBIC ACID	0.654	0.659	0.811	0.973	1.123	1.145

Table 3: Total phenolic compound -Fresh and Photo activated Cow urine

S.NO	SAMPLES (µl/mL)	Absorbance at 765 nm					
		100	200	400	600	800	1000
1.	Fresh COW URINE	0.080	0.168	0.351	0.479	0.605	0.763
2.	Photo activated cow urine	0.065	0.153	0.295	0.366	0.583	0.605
3.	ASCORBIC ACID	0.155	0.227	0.428	0.611	0.712	0.904

Table 4: Susceptibility Test

Samples	Zone of Inhibition level (mm)
Tetracycline	23
Fresh cow urine	25
Photo activated cow urine	22

Table 5: Phytochemical Screening- Fresh and Photo activated Cow urine

Samples	Carbohydrates	Glycosides	Phenol	Proteins and Aminoacids
FCU	+	-	+	+
PAU	+	-	+	+

+ Positive and – Negative result

Fig1: Percentage scavenging of DPPH -Fresh and Photo activated cow urine

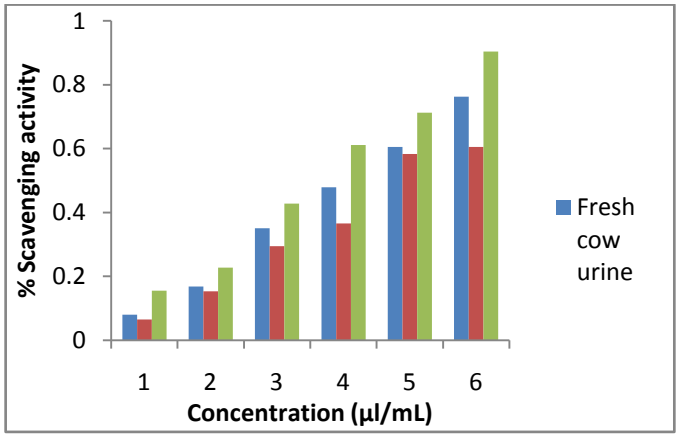

Fig 2: Reducing power Assay-Fresh and photo activated cow urine

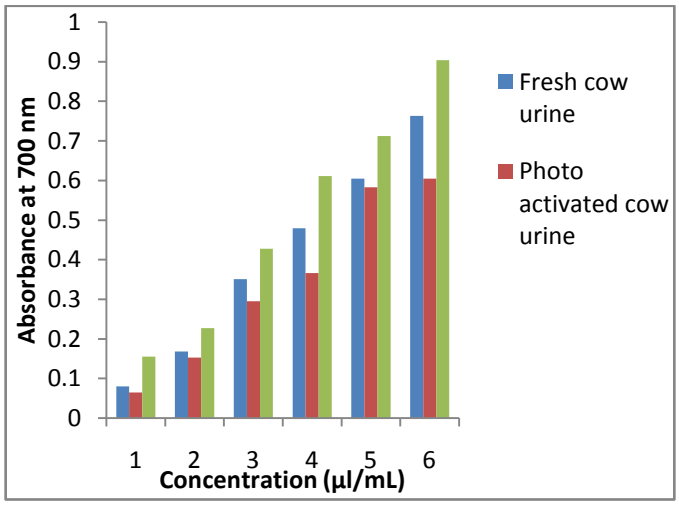

Fig 3: Total phenolic compound -Fresh and Photo activated Cow urine

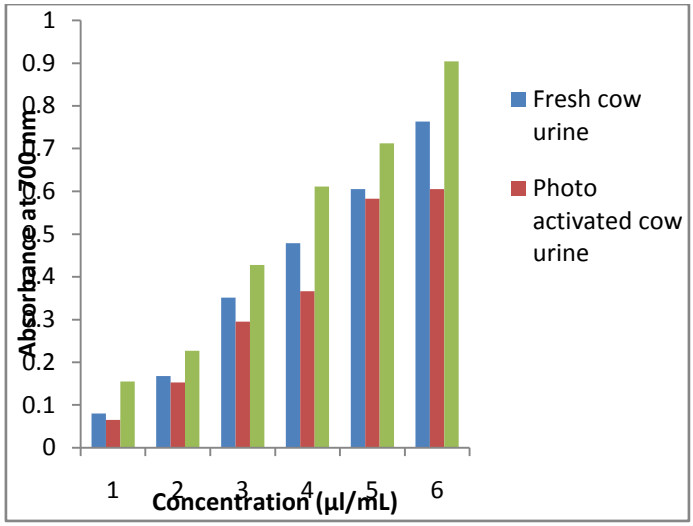

Fig 4 Susceptibility Test

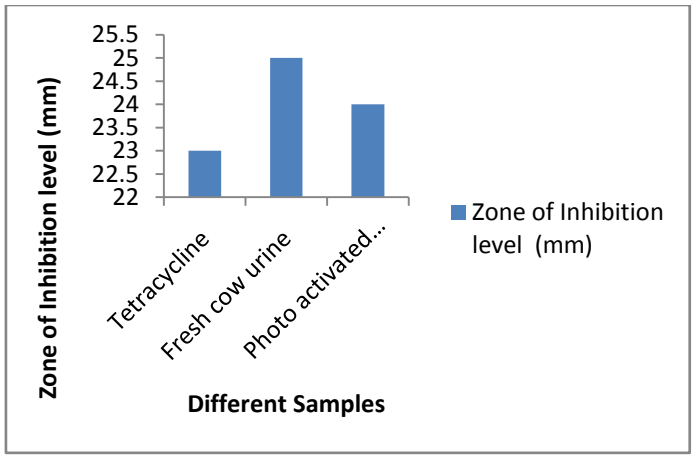

Fig: 5 Antibacterial activity

Bacillus subtilis

Pseudomonas auerogenisa

Klebsiella pneumoniae

RESULTS AND DISCUSSION

Antioxidant Assay

Formation of yellowish color indicates the potent antioxidant behavior of gomutra from free radical scavenging assay. In the free radical scavenging assay, when the DPPH is exposed to antioxidant compounds the purple color of DPPH changed to yellow. The more yellowish color of DPPH observed the greater the antioxidant activity of the tested compounds. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants [8].The percentage of DPPH radical scavenging activity is presented in Table & fig 1. The DPPH radical scavenging activity of the test sample i.e. fresh and photo activated cow urine increases with increasing concentration and found to be more effective. However, activity of test sample was comparable with ascorbic acid. The DPPH radical scavenging activity of the samples increases with increasing concentration due to the presence of phenol as antioxidant chemical[9,10]. Fresh and photo activated cow urine was found to be effective chemical (antioxidant) against free radicals and prevents the oxidation of other chemicals [3].

Reducing power

Reducing power assay method is based on the principle that substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The reductive capability of the sample was found to be 0.413, 0.578, 0.688, 0.847, 0.957 and 1.032. The reductive capability of the cow urine was compared with ascorbic acid for the reduction of the $Fe^{3+}$to Fe^{2+}. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. Table & fig 2 shows the reducing power of fresh and photo activated cow urine [5, 8]. From Table 2 it was found that the reducing power of the samples increased with the increase in concentrations and it was lower than that of ascorbic acid which was used as control. This shows that the fresh and photo activated cow urine possess reducing power capabilities and act as a potent antioxidant against diseases.

Determination of Total Phenolic compounds

The phenolic contents of fresh and photo activated cow urine were evaluated using the Folin-Ciocalteau reagent [10]. The concentration of samples was found to be 0.080, 0.168, 0.351, 0.479, 0.605 and

0.763. The results were tabulated in table and fig 3.

Antimicrobial Activity

The fresh, photo activated and distillate cow urine were analyzed against bacterial pathogens, *Bacillus subtilis, Klebsiellapneumoniae* and *pseudomonas aeruginosa* by well diffusion and pour plate method. Among the three fractions, fresh and photo activated shows better inhibition compared to distillate fractions (Fig 4 & 5). Maximum zone of inhibition was observed in *Klebsiellapneumoniae*(31 & 27mm), *pseudomonas aeruginosa* (28 & 23mm) and *Bacillus subtilis* (25&20mm). The presence of phenols in cow urine may be responsible for bactericidal against pathogens. In fresh and photo activated cow urine having high amount of phenols when compared to distillate fractions may be reason for its better zone inhibition [5]. The fresh and photo activated cow urine was compared with that of standard antibiotics (Tetracycline).The results were shown in fig 6.

Phytochemical Screening for gomutra

Phytochemical screening was performed qualitatively for fresh and photo activated cow urine[11]. In Molisch's test, the reddish violet ring appeared at the junction of two layers which indicates the presence of carbohydrates. The disappearance of the black color was obtained in Borntrager's test, which shows the absence of glycosides. In Lead acetate test the white precipitate was appeared which confirms the presence of phenol compounds. The red color precipitate appeared in Millon's test, which confirms the presence of proteins and free amino acids. The results were tabulated in Table 4.

CONCLUSION

Present study deals with the antioxidant and antibacterial activities of fresh and photo activated cow urine. The antipathogenic activities of cow urine against various pathogenic strains such as *Bacillus subtilis, Pseudomonas auerogenisa*, and *Klebsiellapneumoniae*were studied. The Zone of inhibition was measured and their activities were compared with standard antibiotics (tetracycline). Finally we conclude that the fresh and photo activated cow urine could be a potential source of natural antipathogenic activities and good source of compounds with antioxidant properties. Phenolic contents were evaluated as they are responsible for antioxidant activity. As this study establishes antifungal activity and also evaluate which compounds are responsible for antimicrobial activity.

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