



The investigation of antioxidant power by FRAP test of cow urine (Geer cow)

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Abstract : The study investigates the antioxidant power of cow urine using the Ferric Reducing Antioxidant power (FRAP) assay, a reliable method for measuring the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). Cow urine, key component in traditional medicine, is reputed for its bioactive properties, including its potential to combat oxidative stress. The cow urine and its distillate tested for antioxidant activities exhibited the mentioned activities and comparatively fresh cow urine was found to be better than its distillate. These results indicate that the cow urine has antioxidant activities, which supports the claim of traditional practitioners.

IndexTerms Cow urine, Antioxidant, Free radicals, UV- spectrophotometer, FRAP analysis.

INTRODUCTION

Hospital or health care waste is generally named & popular as biomedical waste. The world health organization defines biomedical waste as ,”Waste generation by health care activities & includes blood, used needles, pharmaceuticals, radioactive materials etc.” The biomedical waste is also known as infectious waste or medical waste or health care waste. According to biomedical waste management & handling rules 1998 of India. Biomedical waste means any waste which is generated during the diagnosis, treatment or immunization of human being or animals or in research activities. In simple words biomedical waste is the waste generated by the medical & health institute/agencies.

Biomedical waste management defines waste management as the practices & procedures or the administration of activities that provide for the collection, source separation, storage, transportation, transfer, processing, treatment & disposal of waste . Biomedical waste management is a routine procedure of hospital administration as prescribed by law .Hospital waste , hospital acquired infection , transfusion transmitted diseases, rising incidence of hepatitis B, HIV & Other diseases, create potential threat of infection, contamination & serious health hazards to doctors, nurses, ward boys, support staff, sanitation workers, rag pickers & other health care workers. Who are regularly exposed to biomedical waste as an occupation hazards as well as general public in the surrounding area .

RESEARCH METHODOLOGY

. Procurement of cow urine

The urine of Gujarati Indian cow known as Geer cow was used in the study. The study was performed after getting a certificate from the Veterinary doctor stating that it is free from diseases. Fresh urine was collected and filtered. Chemo profiling confirmed the presence of protein, urea, uric acid, creatinine, phone, aromatic acids, enzymes like acid phosphatase, alkaline phosphatase, amylase and vitamins [7].

"The BSDT's Ayurved Mahavidyalaya obtain cow urine from its own facility, a well-kept cow shed. The cows there are verified breeds and are monitored closely by a veterinarian"[10].

Antioxidant activity

The antioxidant activity of fresh urine and its distillates was carried FRAP method.

The antioxidant activity of a methanolic extract of *M. Serratulum* and its fractions. The method used is based on the principle of reducing power, where antioxidants can reduce ferric ions (Fe^{2+}) in a reaction mixture.

Different concentrations of the extract and its fractions were prepared in a buffer solution containing potassium ferricyanide. This ferricyanide acts as the oxidizing agent. The reaction mixture was incubated at a specific temperature (50%) for a set time (20 minutes) to allow the antioxidant in the extract to react with the ferricyanide. The reaction was stopped by adding trichloroacetic acid, and the mixture was centrifuged to remove any precipitate. Ferric chloride was added to the supernatant, which reacts with the reduced ferrous ions to form a colored complex. The absorbance of the colored solution was measured at 700 nm using a UV-Spectrophotometer. A higher absorbance indicates a higher reducing power, which is indicative of stronger antioxidant activity. The antioxidant activity of the methanolic extract of *M. serratulum* and its fractions was evaluated using a ferric reducing antioxidant power (FRAP) assay. This assay measures the ability of antioxidants to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}).

The extracts and fractions exhibited significant reducing power, comparable to the reference standard ascorbic acid, indicating their potential antioxidant properties.

FRAP Test Method

Different concentrations of the methanolic extract and its fractions (10-100%) were prepared in 0.2M sodium phosphate buffer (pH 6.6). Reaction Mixture:- 2.5mL of the prepared sample was mixed with 2.5mL of 1% potassium ferricyanide [K₃Fe(CN)₆] solution. The reaction mixture was vortexed to ensure thorough mixing and then incubated at 50°C for 20 minutes using a vortex shaker. 2.5mL of 10% trichloroacetic acid was added to the reaction mixture which was then centrifuged at 3000rpm for 10 minutes to remove precipitated proteins. 2.5 mL of the supernatant was mixed with 2.5mL of deionized water and 0.5mL of 0.1% ferric chloride. The mixture was allowed to develop a yellow to blue colored solution. The absorbance of the colored solution was measured at 700nm using a UV-spectrophotometer. A blank solution (without the sample) was used as a reference. The reducing power of the samples was compared to a standard curve generated using ascorbic acid as a reference standard.

RESULTS AND DISCUSSION

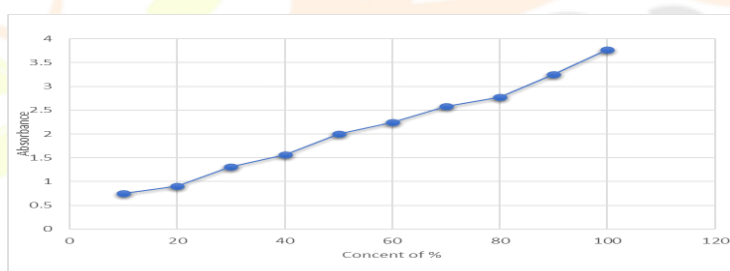
In this study, we aimed to assess the antioxidant potential of cow urine the Ferric Reducing Antioxidant Power (FRAP) assay.

Cow urine samples were collected and stored for 1 to 6 days. The antioxidant capacity of these samples was determined using the FRAP assay. This assay measures the ability of a sample to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺). The absorbance of the resulting Fe²⁺-TPTZ complex was measured at 700 nm using a UV- visible spectrophotometer. A series of diluted cow urine sample (10% to 100%) were prepared from each storage day (1-6). The absorbance of these samples was measured at 700 nm using a UV- visible spectrophotometer. The resulting data were plotted to visualize the antioxidant power over time.

The antioxidant power of fresh cow urine (Day 1) was found to be increasing with dilution. This trend was observed to be consistent on Days 2 and 3 (tab.no.2,3 & graph no. 2,3)

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	0.752	0.908	1.314	1.560	1.997	2.248	2.577	2.774	3.252	3.769

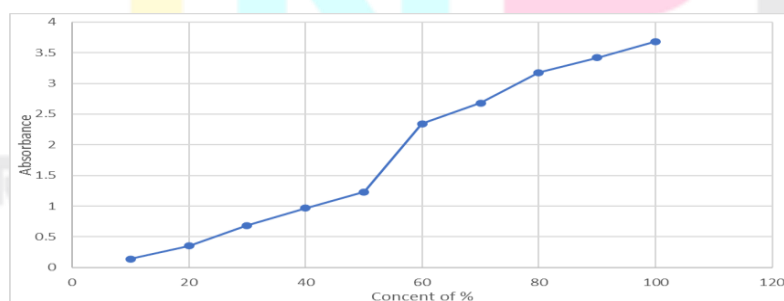
(Tab no. 1)



(Graph no. 1)

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	0.138	0.352	0.688	0.968	1.234	2.342	2.681	3.176	3.419	3.683

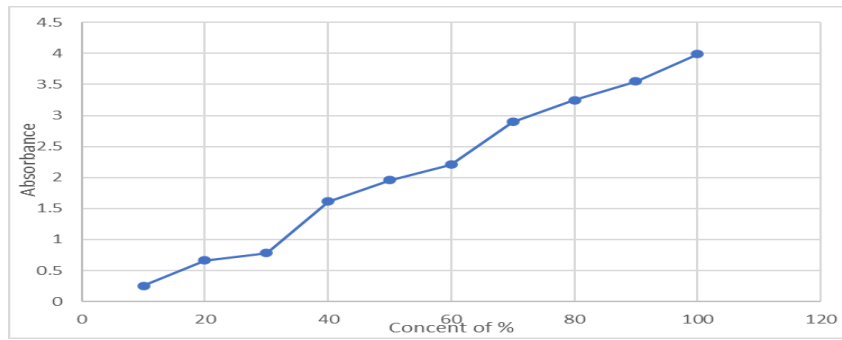
(Tab no. 2)



(Graph no. 2)

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	0.252	0.661	0.781	1.614	1.959	2.205	2.898	3.250	3.550	3.990

(Tab. no. 3)

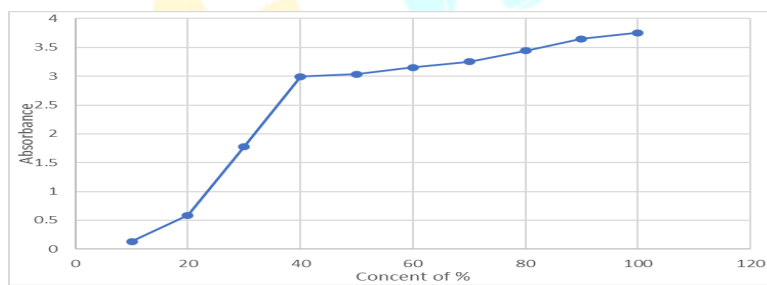


(Graph no. 3)

On day 4, a mixed trend was observed, with lower dilution (10-40%) showing increasing antioxidant power and higher dilutions (50-100%) showing decreasing power.

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	0.130	0.586	1.778	2.996	3.034	3.158	3.258	3.440	3.648	3.753

(Tab no. 4)

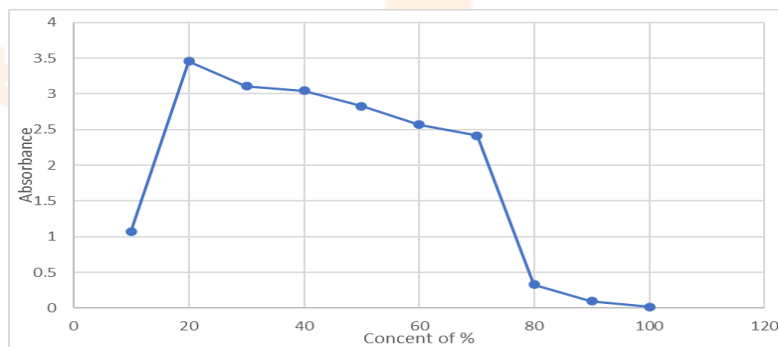


(Graph no. 4)

However, on days 5 and 6, a gradual decline in antioxidant power was observed.

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	1.075	3.431	3.103	3.045	2.826	2.568	2.415	0.327	0.096	0.017

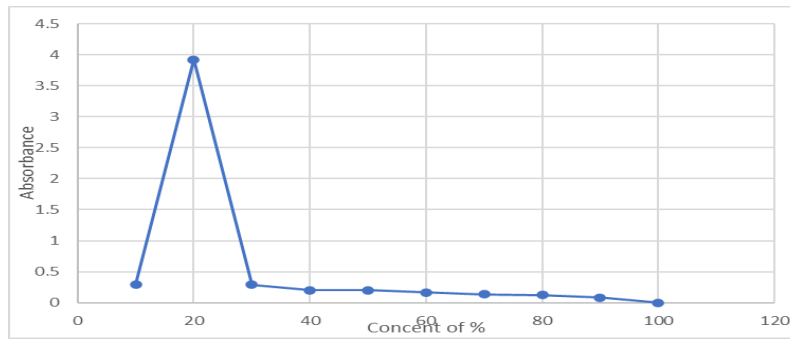
(Tab. no. 5)



(Graph no. 5)

Con. %	10	20	30	40	50	60	70	80	90	100
Abo	0.295	3.922	0.283	0.203	0.200	0.166	0.134	0.123	0.081	0

(Tab no. 6)



(Graph no. 6)

The Ferric Reducing Antioxidants Power (FRAP) assay measures the antioxidant capacity of cow urine by measuring its ability to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) and a pH of 6.6. This reaction produces a blue color that absorbs light at 700 nm. Its absorbance was checked using a UV-spectrophotometer. The antioxidant capacity was measured to investigate the relationship between antioxidant status and stability. Antioxidants are molecules that donate electrons to free radicals to stabilize them. This is found in natural products. Cow urine sample were collected and stored for a duration of 1 to 6 days. The antioxidant capacity of these sample was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. This assay quantifies the ability to a sample to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). A diluted series (10% to 100%) prepared from the sample (1 to 6 days) & the absorbance was checked in UV-spectrophotometer at 700 nm. On 1st to 3rd day antioxidant power increasing with dilute on 4th day mixed trend was absorbance, (10% to 40%) showed increasing antioxidant power & higher dilution. (50% to 100%) shows decreasing power. Gradually on 5 and 6 days, a gradual decline in antioxidant power was observed. from this result from FRAP test we got conclusion that, antioxidant power in urine is only present till 4 days. here are some key points regarding the usage o cow urine concerning its antioxidants properties.

Gomutra (cow urine) believe to have therapeutic properties, including as detoxifier and balancing body energies. its purported antioxidant capacity its often cited in context of promoting overall health Studies have investigated the phytochemical constituents of urine, which may include various bioactive compounds. these substances are thought to contribute antioxidant properties, helping to combat oxidative stress in the body. Global interest in natural antioxidants has driven increased research into the potential applications of animal-derived products, including *Bos taurus* urine, in nutraceutical and pharmaceutical contexts. While some cultures traditionally view *Bos taurus* urine as a remedy with antioxidant properties, scientific understanding of its efficacy and mechanisms of action remains limited. Further rigorous studies are necessary to establish its potentials benefits. Individuals considering the use of *Bos taurus* urine, particularly for medicinal purposes, should exercise caution and consult with healthcare professionals.

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