DISCOVERY

*58(317), May 2022*

# Bioactive Potential of Cow Urine from two Indigenous Cattle Breeds of Southern India

**Kodandoor Sharathchandra[1], Devadiga G. Anitha[1], Shirankallu Soumya[2], Kandikere R. Sridhar[1]***

**To Cite:**
Sharathchandra K, Anitha DG, Soumya S, Sridhar KR. Bioactive Potential of Cow Urine from two Indigenous Cattle Breeds of Southern India. *Discovery*, 2022, 58(317), 428-440

**Author Affiliation:**
[1]Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India.
[2]Department of Microbiology, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India.

***Corresponding author:**
E-mail: kandikere@gmail.com

**Peer-Review History**
Received: 09 March 2022
Reviewed & Revised: 11/March/2022 to 14/April/2022
Accepted: 15 April 2022
Published: May 2022

**Peer-Review Model**
External peer-review was done through double-blind method.



## ABSTRACT

Cow urine is one of the important components of ancient ethnic medicine in the Indian subcontinent. Native cow urine is highly valuable owing to its diet consisting of wild plant species. The composition of urine depends on the cow breed as well as its diet. In Ayurveda, cow urine is used in three forms (raw, sterile and photo-activated urine). All these urine types are endowed with a variety of biochemical components with potent bioactivities. Among the urine of two cow breeds tested (Kasargod Dwarf and Deoni), all types of urine of Deoni possess a higher quantity of total phenolics and tannins, while flavonoid content was higher in Kasargod Dwarf. The ferrous ion-chelation capacity and DPPH radical scavenging activity were higher in all urine samples of Deoni, while the total antioxidant activity was higher in Kasargod Dwarf. Photo-activated urine samples showed the highest antibacterial as well as antifungal activity with minimum inhibition concentration. This study contributes toward the validation of cow urine as a potential source of bioactive compounds and opens up new ways for its applications in human medicine.

**Keywords:** Antimicrobial activity, Biochemical profile, Bioactivity, Cow breed, Photo-activation, Traditional medicine

## 1. INTRODUCTION

The cow (*Bos indicus* Linn.) (Sanskrit, *Kamadhenu*) is one of the most valuable animals in the Indian religious scriptures. The cow urine is a major ingredient of '*Panchagawya*' (meaning, five cow-derivatives), which is capable of treating many diseases and is being used extensively in Ayurvedic medicines (Vasanthi & Venkatalakshmi, 2015; Sattanathan & Venkatlakshmi, 2015; Pant et al., 2019). It is also one of the ingredients in topical lotions, ointments and bath, while it is useful in the preparation of oral related medicines too. There are existing and innumerable examples in ancient medical texts regarding the curative properties of cow urine for various human ailments. Cow urine is composed of water (95%), urea (2.5%), and the remaining mixture of minerals, salts, hormones and enzymes (2.5%). The laboratory analysis of cow urine revealed the presence of many constituents (e.g. minerals: calcium, chloride, iron, magnesium, manganese, nitrogen, phosphate, silicon, sodium, sulfur; organic acids: carbolic, citric malic and succinic acids; vitamins: A, B, C, D, E; enzymes and hormones) (Bhadauria,

2002; Mahajan et al., 2020). It has good germicidal qualities owing to the presence of aurum hydroxide (swarn kshar), calcium, carbolic acid, creatinine, manganese, phenols and urea. It has anti-cancer properties owing to the presence of uric acid, antioxidants and allantoin (Jain et al., 2010; Rachana and Sreepada, 2019; Khushboo et al., 2021).

The origin of many effective drugs can be traced to the prevailing practice of traditional medicines (Saga and Yamaguchi, 2009; Upadhyay et al., 2010). Since ancient times use of cow urine has been mentioned in Ayurvedic texts: *Charaka Samhita, Susruta Samhita* and *Ashtanga Sangraha*. It is recommended orally as well as topically for various infections and is capable to cure about 168 diseases (Rana and De, 2013; Kekuda et al., 2014). The cow has been considered a live dispensary and storehouse of medicines (Jain et al., 2010; Singla and Kaur, 2016). The rural population in India employs native cow urine as a remedy to get rid of many diseases. It is also designated as "*Sanjivani*" or "*Amrita*" in Ayurveda owing to its antibiotic, anti-allergic, antioxidant and antimicrobial potential (Ashara and Shah, 2016).

The uncontrolled use of chemotherapeutic drugs is responsible for breeding hard to control multidrug resistant microbial strains (Minocheherhomji and Vyas, 2014; Nautiyal and Dubey, 2021). Cow urine has the capacity to destroy the toxic effects of medicinal residues without causing damage to the host (Chauhan et al., 2001). In agricultural practices, cow urine serves as an organic fertilizer as well as a potential biopesticide. It has the capacity to boost immunity, eliminate toxic constituents via antioxidants and scavenge free radicals (Jerald et al., 2008; Randhawa and Sharma, 2015). The free radicals are known to cause cell damage by inducing tumor cell growth and also cause aging. Cow urine is well known for its many biological properties (e.g. immunomodulatory, anti-allergic and antimicrobial). In recent years, the increased prevalence of resistance of microbes to the available antimicrobial agents posed serious healthcare implications to society. As a result, there is always a demand for new antimicrobial agents, where natural products are novel sources of bioactive compounds.

Urine samples of two native Indian breed cows (Kasargod Dwarf and Deoni) were taken as the test material to evaluate their composition and bioactive potential. Evaluation of cow urine (fresh, sterile and photo-activated) biochemical profile, antioxidant components, antioxidant potential and antimicrobial activity have been evaluated.

## 2. SAMPLES AND PROCESSING

Two breeds of the cow were selected for urine analysis including Kasargod Dwarf and Deoni. The Kasargod Dwarf is an endangered cattle available in limited regions of Kerala (Kasargod District) and Karnataka (Mangalore and Kodagu Districts) (Fig. 1a). The Deoni is originally from Maharashtra (Latur District), distributed in the northern region of Karnataka (Bidar District) and some have been adapted to the southwestern region of Karnataka (Mangalore District) (Fig. 1b).


**Fig. 1.** Kasargod Dwarf with its calf (a) and Deoni (b).

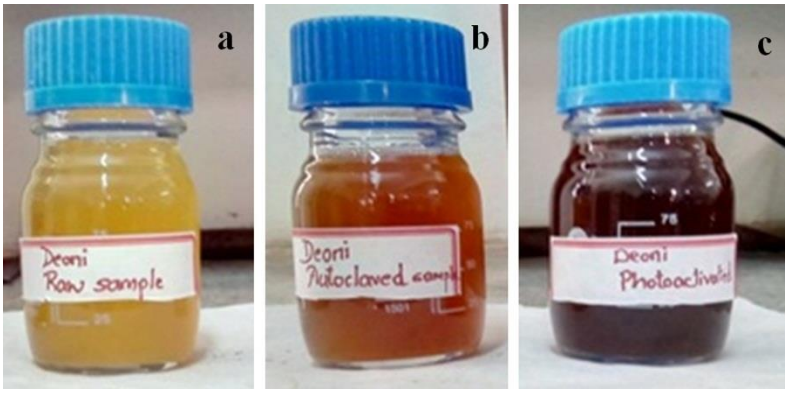


**Fig. 2.** Raw (a), sterile (b) and photo-activated (c) urine samples of the cow breed Deoni.

Both cow breeds are dwarf and high milk-yielding varieties and maintained on the natural diet rather than synthetic feeds. Urine samples of Kasargod dwarf (from Kanyana, Mangalore District) and Deoni (Brahmavara, Udupi District) were collected in wrapped sterile glass bottles thrice in a fortnight period for analysis in the laboratory without exposure to light.

As the urine samples used in traditional medicine in different forms, three sets were maintained after filtering through Whatman #1 filter paper: 1) raw urine (fresh); 2) sterile urine; 3) photo-activated urine (Fig. 1a-c). The raw urine was kept in sterile amber colored bottles. For sterile samples, urine was sterilized in an autoclave (121.5°C for 15 min). For photo-activated urine, urine samples were kept in sunlight for up to 14 days in transparent glass bottles.

## 3. PHYSICOCHEMICAL PROPERTIES

The temperature and pH of raw urine samples were assessed on the spot using Water Analyser (# 371, Systronics, Gujarat, India). Conductivity, total dissolved solids (TDS) and salinity were assessed in the laboratory using the same water analysis kit. Turbidity was determined using the measuring cylinder method, titration method for alkalinity (by strong acid, phenolphthalein and methyl orange indicators), creatinine was carried out by Jaffe's turbidometric method, chlorides by argentometric method, phosphate by stannous chloride method, and sulfate and silicate by molybdosilicate method (APHA, 1998). To gravimetrically estimate the oil and grease content, the urine samples were extracted in petroleum ether using a separatory funnel followed by evaporation of petroleum ether (APHA, 1998).

The raw urine samples of two cow breeds were either medium or pale brown with a slightly bitter taste. Temperature, pH, alkalinity, creatinine and phosphorus contents were higher in Kasargod Dwarf than in Deoni, while the rest of the properties (conductivity, total dissolved solids, salinity, turbidity, chloride, sulfate, silicate, and oil and grease) were higher in Deoni than Kasargod Dwarf (Table 1).

**Table 1.** Physicochemical properties of urine samples of two cow breeds (mean of three replicates).

| | **Kasargod Dwarf** | **Deoni** |
|---|---|---|
| Colour | Medium-brown | Pale-brown |
| Taste | Slightly bitter | Slightly bitter |
| Temperature (°C) | 33.0 | 31.5 |
| pH | 8.33 | 7.8 |
| Conductivity (mS/cm) | 2.03 | 6.20 |
| TDS (mg/l) | 0.92 | 1.99 |
| Salinity (ppt) | 0.48 | 2.17 |
| Turbidity (NTU) | 0.15 | 0.25 |
| Alkalinity (mg/l) | 960.0 | 902.5 |
| Creatinine (mg/l) | 1.13 | 0.29 |



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

| | | |
|---|---|---|
| Chloride (mg/l) | 461.5 | 802.3 |
| Phosphate (mg/l) | 2.08 | 0.56 |
| Sulphate (mg/l) | 0.057 | 0.072 |
| Silicates (mg/l) | 0.013 | 0.095 |
| Oil and grease (%) | 0.06 | 0.32 |

To assess the composition of minerals, the urine samples were lyophilized and the powder has been subjected to the Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (SEM-EDS) method (Ramamurthy and Kannan, 2009).

The carbon content was almost equal in the urine of the two breeds. Potassium, chloride, aluminum and silicate were higher in the urine of Kasargod Dwarf than in Deoni (Table 2), while the rest of the minerals (nitrogen, sulfur, sodium and magnesium) were higher in Deoni than Kasargod Dwarf.

**Table 2.** Mineral composition urine samples *(atomic %)* of two cow breeds.

| | ***Kasargod Dwarf*** | ***Deoni*** |
|---|---|---|
| *C* | *27.04* | *27.06* |
| *N* | *8.25* | *9.82* |
| *K* | *8.42* | *7.51* |
| *S* | *0.66* | *1.29* |
| *Cl* | *7.84* | *1.65* |
| *Na* | *1.83* | *4.57* |
| *Ca* | *0.80* | *0.84* |
| *Mg* | *2.69* | *3.61* |
| *Al* | *0.12* | *BDL* |
| *Si* | *0.44* | *0.19* |

## 4. BIOACTIVE POTENTIAL

### 4.1. Biochemical Profile

Qualitative assay of biochemical constituents of raw, sterile and photo-activated urine samples (alkaloids, saponins, tannins, phenols, triterpenes, flavonoids, anthraquinone glycosides, cardiac glycosides, quinone, carotenoids, phycocyanins and coumarins) were determined adapting standard methodology (Harborne, 1998; Kokate, 1988; Sadashivam and Manickam, 2005). Among the biochemical constituents tested, except for anthraquinone glycosides and Phycocyanins, the rest of the compounds were present in raw, sterile and photo-activated urine samples of both cow breeds (Table 3).

**Table 3.** Qualitative assessment of the biochemical composition of urine samples of two cow breeds (+, detected; ND, not detectable).

| | **Kasargod Dwarf** | | | **Deoni** | | |
|---|---|---|---|---|---|---|
| | **Raw** | **Sterile** | **Photo-activated** | **Raw** | **Sterile** | **Photo-activated** |
| Alkaloids | + | + | + | + | + | + |
| Saponins | + | + | + | + | + | + |
| Tannins | + | + | + | + | + | + |
| Phenols | + | + | + | + | + | + |
| Triterpenes | + | + | + | + | + | + |
| Flavonoids | + | + | + | + | + | + |
| Anthraquinone glycosides | ND | ND | ND | ND | ND | ND |
| Cardiac glycosides | + | + | + | + | + | + |
| Quinone | + | + | + | + | + | + |



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

| | | | | | | |
|---|---|---|---|---|---|---|
| Carotenoids | + | + | + | + | + | + |
| Phycocyanins | ND | ND | ND | ND | ND | ND |
| Coumarins | + | + | + | + | + | + |

**4.2. Antioxidant Components**

The content of total phenolics was assessed using Folin Ciocalteu's method (Mc Donald et al., 2001) and expressed as standard gallic acid equivalent (mg GAE/ml). The tannin content was determined by Folin-Ciocalteau's method (Singh et al., 2012) and expressed as standard tannic acid equivalent (mg of TAE/ml). The aluminum chloride spectrophotometric method of Chang et al. (2002) was and expressed in terms of standard quercetin equivalent (mg QE/ml).

Total phenolics content in raw urine of Kasargod Dwarf as well as Deoni showed the highest followed by photo-activated and sterile urine (Fig. 3a). The tannin content was highest in photo-activated urine of Kasargod Dwarf as well as Deoni followed by raw and sterile urine samples (Fig. 3b). The flavonoids content was highest in raw urine samples of Kasargod Dwarf as well as Deoni followed by photo-activated and sterile urine samples (Fig. 3c).

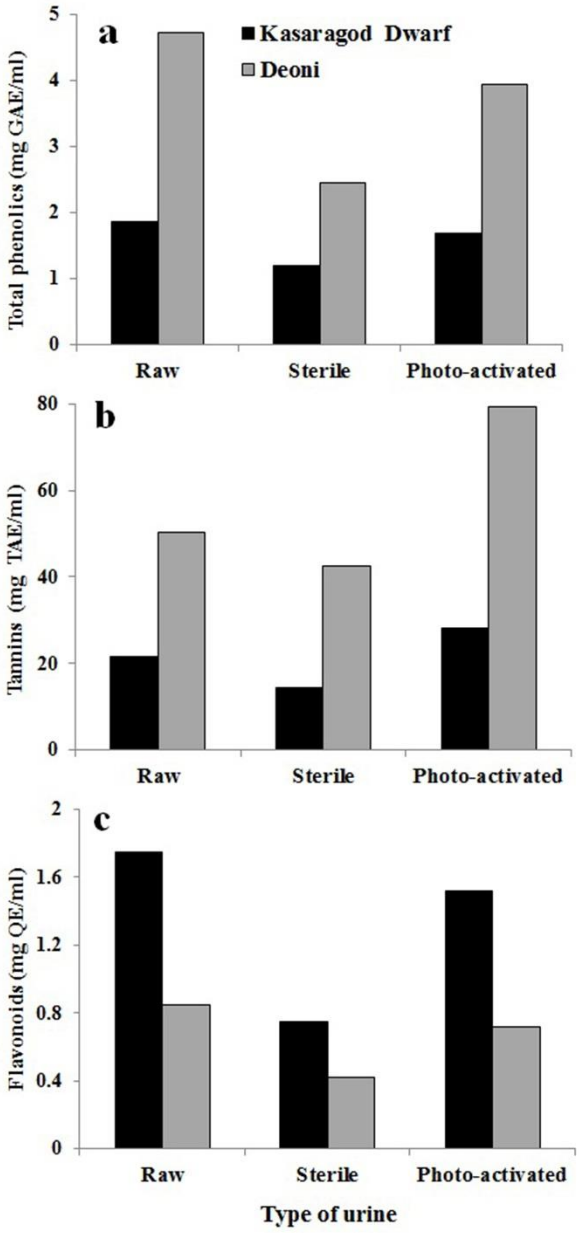


**Fig. 3.** Total phenolics, tannins and flavonoids of urine samples (mean of three replicates).

**4.3. Antioxidant Activity**

The total antioxidant activity of urine samples was analyzed by the protocol by (Prieto et al., 1999) and the activity was expressed using standard ascorbic acid equivalent (mg AAE/ml). Ferrous ion-chelation capacity was assessed by the procedure by Hsu et al. (2003) and its capacity was expressed in percent (absorbance at 700 nm). The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radial-scavenging activity was estimated by the standard protocol (Barreira et al., 2008) and expressed in percent (absorbance at 517 nm).

The total antioxidant activity was highest in photo-activated urine samples of Kasargod Dwarf as well as Deoni followed by sterile and raw urine (Fig. 4a). So also for the ferric ion-reducing capacity and the DPPH radical-scavenging activity (Fig. 4b, c).

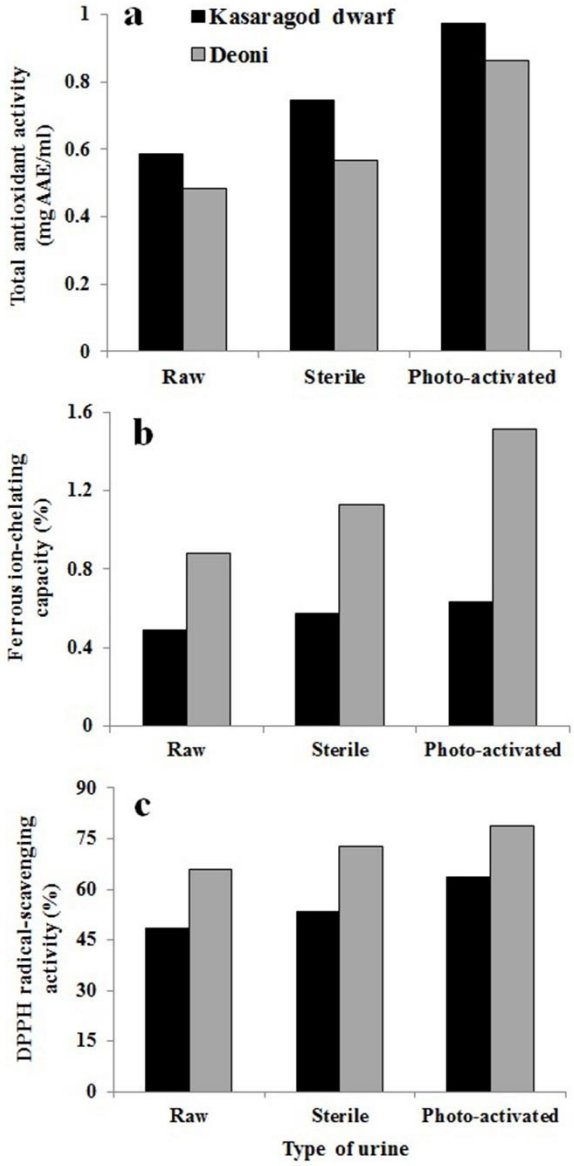


**Fig. 4.** Total antioxidant activity, ferrous ion-chelation capacity and the DPPH radical-scavenging activity of urine samples (mean of three replicates).

**4.4. Antimicrobial Potential**

To assess the antimicrobial potential of three types of urine samples, standard bacterial cultures (*Staphylococcus aureus* MTCC-7443 and *Klebsiella pneumoniae* MTCC-139) as well as standard fungal cultures (Candida albicans-MTCC 183 and Neurospora crassa MTCC-260), were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. For the antibacterial assay, the inoculum of bacteria was prepared by mixing a loop full of culture broth (24 hr old culture in Mueller Hinton Broth at 37°C on a shaker incubator) to yield approximately $1.0 \times 10^7$-$1.0 \times 10^8$ CFU/ml using a spectrophotometer (25% transmittance at 660 nm). For



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

the antifungal assay, the inoculum of test strains was prepared as above by mixing a loop full of fungal culture from 48 hr old culture plate in 5 ml of sterile Potato Dextrose Broth in screw cap vials and incubated (up to 48 hr at 28°C) in a shaker incubator.

An antimicrobial assay was carried out using the disc diffusion technique (Vardarunler et al., 2003). Streptomycin was used as a standard antibiotic against bacteria, while nystatin was against fungi. The minimum inhibition concentration (MIC) of samples was tested against the bacterial isolates by microdilution method using a 96 well microtitre plate (using ELISA plate reader-Thermo Fisher, Scientific, Germany). Mueller-Hinton Broth was used as medium and varying concentration of the cow urine samples 50, 25, 15 and 10 µg/ml in ethanol was prepared to test the antibacterial activity. The 96 well titre plate was seeded with the media (200 µl) containing the test organism (OD equivalent to 0.5 Mc Farland). Then 100 µl of each prepared cow urine sample was poured into different wells and incubated (24 hr, 37ºC). After incubation (24 hr), the MIC was calculated as the lowest concentration resulting in inhibition of the growth of tested bacteria. For the antifungal activity of the cow urine samples against the fungal isolates, a similar methodology was followed as above where, potato dextrose broth was used as medium and after the addition of the fungal inoculum and test samples, 96 well microtitre plate was incubated (48 hr, 28ºC).

Among the raw, sterile and photo-activated samples, the photo-activated samples showed the highest bacterial inhibition activity in Kasargod Dwarf as well as Deoni (Table 4). However, inhibition was lesser than the streptomycin. The MIC was the lowest in photo-activated urine samples against test bacteria in both cow breeds indicating the importance of the use of photo-activated urine in medicine (Table 4).

**Table 4.** Antibacterial activity of cattle urine samples of two cow breeds with minimum inhibition concentration (mean of three replicates).

| | **Urine** | **Diameter of zone of inhibition (mm)** | |
|---|---|---|---|
| | | ***Staphylococcus aureus*** | ***Klebsiella pneumoniae*** |
| **Extent of inhibition (mm)** | | | |
| *Kasargod Dwarf* | Raw | 12.0 | 10.5 |
| | Sterile | 10.0 | 8.5 |
| | Photo-activated | 19.5 | 16.0 |
| *Deoni* | Fresh | 12.5 | 11.0 |
| | Sterile | 11.0 | <8.0 |
| | Photo-activated | 18.0 | 15.5 |
| | Streptomycin | 30.0 | 24.0 |
| **Minimum inhibition concentration (µg/ml)** | | | |
| *Kasargod Dwarf* | Raw | 25.0 | 50.0 |
| | Sterile | 75.0 | 75.0 |
| | Photo-activated | 15.0 | 10.0 |
| *Deoni* | Fresh | 50.0 | 75.0 |
| | Sterile | 100.0 | 125.0 |
| | Photo-activated | 15.0 | 25.0 |

Similar to the antibacterial activity, the antifungal activity was also highest in photo-activated urine samples of both cow breeds (Table 5). However, it was below the nystatin. The MIC was highest in Kasargod Dwarf in raw as well as sterile urine samples against *Candida albicans*, while it was only in sterile samples against *Neurospora crassa* (Table 5). The MIC against fungi tested was the lowest in photo-activated urine derived from Kasargod Dwarf as well as Deoni.



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

**Table 5.** Antifungal activity of cattle urine samples of two cow breeds with minimum inhibition concentration (mean of three replicates).

| | Urine | Diameter of zone of inhibition (mm) | |
|---|---|---|---|
| | | *Candida albicans* | *Neurospora crassa* |
| **Inhibition (mm)** | | | |
| *Kasargod Dwarf* | Raw | 10.5 | 12.5 |
| | Sterile | 9.0 | <7.0 |
| | Photo-activated | 16.0 | 14.5 |
| *Deoni* | Fresh | 11.0 | 12.0 |
| | Sterile | <7.0 | 9.0 |
| | Photo-activated | 16.0 | 20.0 |
| | Nystatin | 29.0 | 25.0 |
| **Minimum inhibition concentration (µg/ml)** | | | |
| *Kasargod Dwarf* | Raw | 75.0 | 50.0 |
| | Sterile | 75.0 | 100.0 |
| | Photo-activated | 15.0 | 20.0 |
| *Deoni* | Fresh | 100.0 | 75.0 |
| | Sterile | 125.0 | 75.0 |
| | Photo-activated | 25.0 | 15.0 |

## 5. DISCUSSION

An innumerable number of metabolites are obtained directly or indirectly from plant, animal and microbial sources and are known to possess therapeutic potential. These metabolites serve either individually or in combination to prompt remedies against many threatening lifestyle diseases. Among the several animal-derived products, cow urine has been considered more precious owing to its valuable therapeutic potential (Jerald et al., 2008). A combination of cow products (urine, dung, milk, curd and ghee) in a specific proportion is called *Panchagavya*, which is one of the prime constituents in many Ayurvedic medicinal preparations in India owing to its therapeutic significance.

### 5.1. Physicochemical Composition

The physicochemical and mineral composition varies among cow breeds and the medicine preparation will be usually carried out with the urine derived from native cows. Native cows fetch their diet in wild (e.g. grasslands and forests), thus its urine will have many bioactive components from plant sources that are useful in medicinal preparations. Ashara and Shah (2016) reported that cow urine consists of many minerals (Ca, Mg, K, Na and $SO_4$), urea, allantoin, chloride, coproporphyrin, and uroporphyrin, creatinine and uric acid. Apte and Balachandram (2002) reported the presence of estrogens, corticosteroids and keto-steroids in fresh cow urine. Apart from these, several vitamins (A, B, C, D and E) as well as minerals, were also reported in cow urine (Jain et al., 2010). The present study justified earlier reports by reporting varied physicochemical characteristics and minerals in the urine of two cow breeds (see Tables 1 and 2)

### 5.2. Bioactive Potential

Cow urine will be used in Ayurveda in either raw, sterile or photo-activated form depending on the nature of medicine and therapy. Qualitative biochemical analysis of raw, sterile and photo-activated urines showed a variety of constituents that are valuable in therapy as antioxidants and hormones (see Table 3). Many of these constituents are commonly found in plants as well as mushrooms too (Soans, 2018; Dattaraj et al., 2020), which supports the view that native cow urine possesses therapeutically valuable biochemical constituents.

According to Venkatesh et al. (2011), the cow urine extract of a sea moss (*Kappaphycus alavaerzii*) showed the presence of terpenoids, flavones, coumarin, tannin and inulin. Similarly, 20 days of extract of neem leaves (*Azadirachta indica*) in cow urine showed the presence of flavonoids, alkaloids, quinines, coumarins and tannins along with minerals like silver, traces of gold and



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Na/K (4:1) (Rajapandiyan et al. (2011). Herbal preparation by the traditional healers '*Mandsaur*' using cow urine along with plants such as *Gymnema sylvestre* (Asclepiadaceae) and *Momordica charantia* (Cucurbitaceae) in the treatment against diabetes.

Some studies revealed the immunomodulatory, antioxidant and antimicrobial properties of cow urine (Gosavi et al., 2011; Vinotha et al., 2020). Aurum hydroxide is known to improve immunity, while the allantoin stimulates wound healing. Early morning voided cow urine is known to possess a maximum quantity of uric acid and allantoin, thus their antioxidant properties enable the treatment of cancer (Randhawa and Sharma, 2015). Cow urine reduces apoptosis in lymphocytes leading to enhance rate of survival. This property has been ascribed to the free radical-scavenging activity of components in the urine, which is also responsible for slowing down the process of aging (Achliya et al., 2004; Badadani et al., 2007). Thus, the administration of a specific dose of cow urine helps in balancing the antioxidant components desirable to cure immunological diseases. Raw urine of Kasaragod Dwarf as well as Deoni, possess more total phenolics and flavonoids, while photo-activated urine consists of more tannins justifying the presence of antioxidant components in cow urine (see Fig. 3). The total antioxidant activity, DPPH radical-scavenging activity and ferrous ion-chelation capacity were highest in photo-activated urine of both cow breeds validating the antioxidant potential of cow urine (see Fig. 4).

Cow urine is known to increase the activity of gonadotropin-releasing hormone (GnRH) conjugate with serum albumin and zinc (Shah et al., 2011; Minocheherhomji and Vyas, 2014; Sharma et al., 2020). Praba et al. (2015) reported that fresh and photo-activated cow urine show antioxidant activity against free radicals and also possesses a high ferrous ion-reducing capacity. The distillate of cow urine was found to possess high total antioxidant activity mainly due to the presence of volatile fatty acids (Krishnamurthi et al., 2004; Atashi et al., 2015; Archana and Vijayalakshmi, 2018). Distillate of cow urine acts as an activity enhancer by facilitating the availability of bioactive molecules (Wate et al., 2011). Cow urine is effective against certain cardiac problems, kidney disorders, indigestion, stomach ache, edema, skin disease, epilepsy, anemia, constipation and respiratory diseases (Pathak and Kumar, 2003; Rachana et al., 2016). Research has been performed to eliminate the odor as well as pathogenic bacteria from cow urine prior to prescribing or use in medicinal preparations. One of the attempts called '*Arka-kalpana*' is a process that is followed to eliminate odor and pathogens in cow urine (Yadav and Thakare, 2013). The shelf life of cow urine is about five years, which facilitates to preservation of the urine for future applications (Mohanty et al., 2014).

### 5.3. Antimicrobial Potential

According to Kumar (2013), the purified cow urine shows a high antibacterial property. Aruna and Padmapriya (2016) also reported that fresh cow urine is highly effective against bacterial strains at low concentrations. Charmi et al., (2011) studied the antibacterial activity of fresh cow urine, which was more active than photo-activated urine due to the presence of certain volatile and non-volatile components. However, in Kasargod Dwarf as well as Deoni, the photo-activated urine showed the highest antibacterial activity with the least MIC (see Table 4). This justifies the use of photo-activated cow urine in some medicinal preparations in Ayurveda. It is predicted that the hydrolytic cow urine with amino acids as well as urinary peptides enhances the bactericidal activity owing to cell surface hydrophobicity (Badadani et al., 2007; Randhawa and Sharma, 2015). The photo-activation and long-term storage of urine lead to the increase or development of some reactive compounds (e.g. amines, formaldehyde, ketones and sulfinol), which enhances the antibacterial potential (Turi et al., 1997; Manjramkar et al., 2019). According to Upadhyay et al. (2010), the photo-activated cow urine along with essential oil becomes more toxic to several pathogenic bacteria. Praba et al. (2015) showed that fresh and photo-activated cow urine is a potential source of natural anti-pathogenic as well as antioxidant components. Edwin et al. (2008) have demonstrated that fresh cow urine possesses better antioxidant as well as antibacterial activity compared to its distillate. Cow urine is also more effective against the growth of fungal pathogens (Kumar, 2013; Jandaik et al., 2015).

In Uttarakhand of India, cow urine is used to save honey bees from the attack of bacterial diseases during their rearing (Mohanty et al., 2014). This treatment has also facilitated the rapid recovery of infected combs along with the growth promotion of brood and enhanced the efficiency of the worker bees. Thus, this practice served as a prospective ecofriendly measure in the management of honey bees of European foul brood (EFB) from a serious bacterial disease, which is prevalent worldwide.

Similar to antibacterial activity, the cow urine also showed effective inhibition of yeast and filamentous fungi, whereas photo-activated urine of Kasargod Dwarf as well and Deoni showed maximum inhibition with the least MIC (see Table 5). In addition to antioxidant activities, cow urine also possesses the capability to inhibit drug-resistant bacterial and fungal strains (Bristow et al., 1992; Arunkumar et al., 2010).



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

**5.4. Agricultural Potential**

Cow urine has several agricultural implications such as liquid manure, plant growth enhancer and biopesticide, which facilitates to follow organic farming. Some of the products have been created from cow urine to use as biofertilizers as well as pesticides (Dhama et al., 2005; Yadav and Lourduraj, 2005). According to recent studies, cow urine is effective against pests and possesses larvicidal potential, which could be developed in combination with herbal preparations (Ahirwar et al., 2010; Chawla, 2010).

Cow urine is known to boost the yield of annual ryegrass with increased nitrogen in the soil and decreased nitrogen fixation (up to 10%) in clovers during the winter season (Saunders, 1982). Total nitrogen content in cow urine ranges between 6.8 and 21.6 g/l, which is equivalent to 69% in urea (Bristow et al., 1992). Urine has increased the nitrogen and potassium concentration of ryegrass, while only potassium in clover. Increased growth of pasture in the urine-fed spots has been evidently owing to high nitrogen and other essential elements in the urine (e.g. potassium and sulfur). As the shelf life of cow urine is about five years (Mohanty et al., 2014), which is a boon to eco-friendly agricultural practices in the future.

## 6. CONCLUSIONS

Cow urine has been considered an important ingredient in ethnic medicinal systems of the Indian subcontinent. The last few decades have witnessed an incredible development in the therapy of cow urine owing to increased drug-resistant microbes. Variations in physicochemical composition, biochemical profile, antioxidant components, antioxidant potential and antimicrobial activity of urine (raw, sterile and photo-activated) samples of two cow breeds (Kasargod Dwarf and Deoni) have been reported. Urine samples of both cow breeds showed a variety of biochemical components, antioxidant compounds and antioxidant activity. In addition, they were capable of inhibiting bacteria as well as fungi. It is possible to sterilize urine samples using Millipore filters, which stand as another variable without loss of bioactive principles and eliminates pathogens in the urine samples. The cow urine differs in quality and quantity of physicochemical components, bioactive principles, antioxidant activities and antimicrobial potential. Hence, various cow urine could be tested as well as preserved by advanced preservation methods without the loss of bioactive components to formulate appropriate medicine to combat human ailments. There is wide scope to use cow urine along with plant materials in various formulations to tackle many lifestyle diseases. There is a need to systematically validate the claims of cow urine's ability to cure many diseases through *in vitro* as well as *in vivo* approaches.

**Acknowledgements**

The authors are indebted to Mangalore University for encouragement to perform this study and the Department of Biosciences for extending the necessary laboratory facilities. We are thankful to Dr. S. Mahadevakumar, Karnataka State Open University, Mysore for his helpful suggestions.

**Funding**

This study has not received any external funding.

**Conflicts of interests**

The authors declare that there are no conflicts of interests.

**Data and materials availability**

All data associated with this study are present in the paper.

## REFERENCES AND NOTES

1. Achliya, G.S.; Meghre, V.S.; Wadodkar, S.G.; Dorle, A.K. Antimicrobial activity of different fractions of Cow Urine. *Ind. J. Nat. Prod. Resour.* 2004, 20, 14–16.
2. Ahirwar, R.M.; Gupta, M.P.; Banerjee, S. Field efficacy of natural and indigenous products on sucking pests of Sesame. *Ind. J. Nat. Prod. Resour.* 2010, *1*, 221–226.
3. APHA, *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. American Public Health Association Inc., Washington DC, 1998.
4. Apte; Bhalchandram. Analysis of Gomutra and its possible therapeutic implications. 5th International Congress on traditional Asian Medicine, Halle (Saale), 2002, pp 18–24.
5. Archana, I.; Vijayalakshmi, K. Antioxidant potential of phloroglucinol - An *in vitro* approach. *Inter. J. Pharmaceutical Sci. and Res.* 2018, *9*, 2947–2951.
6. Aruna, R.; Padmapriya, S.S. Effect of antimicrobial activity of indigenous cow urine against bacterial fish pathogens. *Res. J. Sci. Technol.* 2016, *8*, 139–141.

7. Arunkumar, S.; Methuselvam, M.; Rajasekaran, R. Antimicrobial activities of cow urine distillate against some clinical pathogens. *Global J. Pharmacol.* 2010, *4*, 41–44.
8. Ashara, K.; Shah, K. Cow's urine: An incredible aqueous phase. *Global J. Biotechnol. Biochem.* 2016, *11*, 145–152.
9. Atashi, F., Modarressi, A.; Pepper, M.S. The role of reactive oxygen species in mesenchymal stem cell adipogenic and osteogenic differentiation - A review. *Stem Cells Dev.* 2015, *24*, 1150–1163.
10. Badadani, M.S.; Babu, S.V.; Shetty, K.T. Optimum conditions of autoclaving for hydrolysis of proteins and urinary peptides of prolyl and hydroxyprolyl residues and HPLC analysis. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci*. 2007, *847*, 267–274.
11. Barreira, J.C.M.; Ferreira, I.C.F.R.; Oliveira, M.B.P.P.; Pereira, J.A. Antioxidant activity and bioactive compounds of ten Portuguese regional and commercial almond cultivars. *Food Chem. Toxicol*. 2008, *46*, 2230–2235.
12. Bhadauria, H. Cow urine - A magical therapy. Vishwa Ayurveda Parishad. *Int. J. Cow Sci.,* 2002, *1*, 32–36.
13. Bristow, A.W.; Whitehead, D.C.; Cockburn, J. E. 1992, Nitrogenous constituents in the urine of cattle, sheep and goats. *J. Sci. Food Agric.* 1992, *59*, 387–394.
14. Chang, C.; Yang, M.; Wen, H.; Chern, J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*. 2002, *10*, 178–182.
15. Charmi P.S.; Patel, D.M.; Dhami, P.; Kakadia, J.D. *In vitro* screening of antibacterial activity of cow urine against pathogenic human bacterial strains. *Int. J. Curr. Pharmaceut. Res.* 2011, *3*, 91–92.
16. Chauhan, R.S.; Singh, B.P.; Singhal, L.K. Immunomodulation with Kamdhenu ark in mice. *J. Immunol. Immunopathol.* 2001, *71*, 89–92.
17. Chawla, P.C. Risorine - A novel CSIR drug curtails TB treatment, *CSIR News* - March, 20, 2010, 52–60.
18. Dattaraj, H.R.; Sridhar, K.R.; Jagadish, B.R.; Pavithra, M. Bioactive potential of the wild edible mushroom *Ramaria versatilis*. *Stud. Fungi* 2020, *5*, 73–83.
19. Dhama, K.; Rajesh, R.; Chauhan, R.S.; Simmi, T. Pahchagavya (cowpathy): An overview. *Int. J. Cow Sci*. 2005, *1*, 1–15.
20. Edwin, J., Sheej, E.; Vaibhav, T.; Rajesh, G. Emmanuel T, Antioxidant and antimicrobial activities of cow urine. *Global J. Pharmacol.* 2008, *2*, 20–22.
21. Gosavi, D.D.; Sachdev, D.; Salwe, K. Immunomodulatory and antioxidant effect of Gomutra ark in rats. *J. MGIMS.* 2011, *16*, 37–41.
22. Harborne, J.B. *Phytochemical Methods*, 3rd Edition, Chapman & Hall, London. 1998.
23. Hsu, C.L.; Chen, W.; Weng, Y.M.; Tseng, C.Y. Chemical composition, physical properties and antioxidant activities of yam flours as affected by different drying methods. *Food Chem*. 2003, *83*, 85–92.
24. Jain, N.K.; Gupta, V.B.; Garg, R.; Silawat, N. Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India - A survey. *Int. J. Green Pharm.* 2010, *4*, 29–35.
25. Jandaik, S.; Thakur, P.; Kumar, V. Efficacy of cow urine as plant growth enhancer and antifungal agent. *Adv. Agric.* 2015, *22*, 1–7.
26. Jarald, E.; Edwin, S.; Tiwari, V.; Garg, R.; Toppo E. Antioxidant and antimicrobial activities of cow urine. *Glob. J. Pharmacol*. 2008, *2*, 20–22.
27. Kekuda, T.R.; Vivek, M.N.; Manasa, M.; Kambar, Y.; Nawaz, A.N.; Raghavendra, H.L. Antifungal effect of cow urine extracts of selected plants against *Colletotrichum capsici* isolated from anthracnose of chilli. *Int. J. Agri. Crop. Sci*. 2014, *7*, 142–146.
28. Khushboo, Kumari, M.; Kalotra, N.; Giri, A. Evaluation of physico-chemical and antioxidant properties of dairy cow, goat and buffalo urine in two different seasons in a sub-tropical region of India. *Ind. J. Animal Res.* 2021, *55*, 25–30.
29. Kokate, C.K. *Practical Pharmacognosy*, 2nd Edition, Vallabh Prakashan, New Delhi, 1988.
30. Krishnamurthi, D.; Dutta, S.; Sivanesan, D.; Chakrabarti, T. Protective effect of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals. *Biomed. Environ. Sci.* 2004, *17*, 247–256.
31. Kumar, S. 2013, Analysis of cow's urine for detection of lipase activity and anti-microbial properties. *J. Pharm, Biol. Sci.* 7, 01–08.
32. Mahajan, S.P.; Chavan, S.A.; Shinde, S.A.; Narkhede, M.B. Miraculous Benefits of Cow Urine: A Review. *J. Drug Deliv. Therapeut.* 2020, 10, 275–281.
33. Manjramkar, A.J.; Deshmukh, V.V.; Waghamare, N.; Vaidya, M.S. Assessment of antimicrobial properties of cow urine distillates. *Int. J. Curr. Microbiol. App. Sci.* 2019, *8*, 2556–2565.
34. Mc Donald, S.; Prenzler, P.D., Autolovich, M.; Robards, K. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. 2001, *73*, 73–84.
35. Minocheherhomji, F.P.; Vyas, B.M. Study of the anti-microbial activity of cow urine and medicinal plant extracts on pathogenic human microbial strains. *Int. J. Adv. Pharm. Biol. Chem.* 2014, *3*, 836–840.
36. Mohanty, I.; Senapati, M.R.; Jena, D.; Palai, S. Diversified uses of cow urine. *Int. J. Pharm. Acad. Sci.* 2014, *6*, 20–22.




ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

37. Nautiyal, V.; Dubey, R.C. FT-IR and GC-MS analysis of potential bioactive compound of cow urine and its antibacterial activity. *Saudi J. Biol. Sci.* 2021, *28*, 2432–2437.
38. Pant B, Joshi S, Manandhar C, Manandhar S, Baidya S. Efficacy testing of eco-friendly and commercial antiviral products for the management of Zucchini Yellow Mosaic Virus. *Discovery*, 2019, 55(285), 484-489
39. Pathak, M.L.; Kumar, A. Gomutra a descriptive study. *Sachitra Ayurveda* 2003, *7*, 81–84.
40. Praba, S.L.; Gnanasaraswathi, S.; Jesudoss, R.P.; Ramya, N.; Devi, S.N. Potential source of fresh and photoactivated gomutra for study of antioxidant and antipathogenic activities against various pathogens. *Asian J. Pharm. Clin. Res*. 2015, *8*, 459–462.
41. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal. Biochem*. 1999, *269*, 337–341.
42. Rachana, B.; Sreepada, K.S. Antioxidant and anti-inflammatory activities of cow urine from Malnad Gidda - An indigenous breed. *Int. J. Pharm. Sci. Res.* 2019, *10*, 612–18.
43. Rachana, B.; Sreepada, K.S.; Hegde, K. Protective effects of urine from Malanad Gidda an indigenous cow breed on paracetamol treated Wistar rats. *J. Free Radic. Antiox.* 2016, *143*, 426–435.
44. Rajapandiyan, K.; Shanthi, S.; Murugan, A.M.; Alagu, Muthu, G.; Ranjit, S.A.J.A. *Azadirachta indica* - Cow urine extract, a novel controlling agent towards clinically significant multidrug resistant pathogens. *J. Appl. Pharmaceut. Sci*. 2011, *1*, 107–113.
45. Ramamurthy, N.; Kannan, S. SEM-EDS analysis of soil and plant (*Calotropis gigantea* Linn.) collected from an industrial village, Cuddalore Dt., Tamil Nadu, India. Roman. *J. Biophys*. 2009, *19*, 219–226
46. Rana, R.; De, S. *In vitro* antimicrobial screening of cow urine-a potential natural antimicrobial agent. *Int. J. Bioassay*s 2013, *2*, 436–439.
47. Randhawa, G.K.; Sharma, R. Chemotherapeutic potential of cow urine: A review. *J. Intercult. Ethnopharmacol.* 2015, *4*, 180–186.
48. Sadashivam, S.; Manickam, A. *Biochemical Methods*, 3rd Edition, New age International publishers, Kolkata, India, 2008.
49. Saga, T.; Yamaguchi, K. History of Antimicrobial agents and resistant. *Jap. Med. Assoc. J.* 2009, *52*, 103–108.
50. Sattanathan G, Venkatlakshmi S. Efficacy of different breeds of cow urine distillate on growth and food utilization Of Indian Major Carp, *Labeo Rohita* (Hamilton) Fingerlings. *Species*, 2015, 14(46), 169-185
51. Saunders, W.H.M. Effects of cow urine and its major constituents on pasture properties. *New Zealand J. Agric. Res.*, 1982, *25*, 61–68.
52. Shah, C.P.; Patel, D.M.; Dhami, P.D.; Kakadia, J.; Bhausar, D.; Vachani, U.D.; Trivedi, M.N.; Joshi, V.J. *In vitro* screening of antibacterial activity of cow urine against pathogenic human bacterial strains. *Int. J. Curr. Pharmaceut. Res*. 2011, *3*, 91–92.
53. Sharma, K.; Kaur, S.; Kumar, N. Cow urine prominence to humanity. *J. Pharmacog. Phytochem*. 2020, *9*, 459–465.
54. Singh, R.; Verma, P.K.; Singh, G. Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium. J. Intercult. Ethnopharmacol*. 2012, *1*, 101–104.
55. Singla, S.; Kaur, S. Biological activities of cow urine - An Ayurvedic elixir. *Eur. J. Pharma. Med. Res.*, 2016, *3*, 118–124.
56. Soans, J.C. Bioactive Components and Antioxidant Attributes of Traditionally Edible Banana Inflorescence (*Musa paradisiaca*). M.Sc. Dissertation, Food Science and Nutrition, Department of Biosciences, Mangalore University, Mangalore, India, 2018.
57. Turi, M.; Turi, E.; Koljalg, S.; Mikelsaar, M. Influence of aqueous extracts of medicinal plants on surface hydrophobicity of *Escherichia coli* strains of different origin. *APMIS* 1997, *105*, 956–962.
58. Upadhyay, R.K.; Dwivedi, P.; Ahmad, S. Antimicrobial activity of photo-activated cow urine against certain pathogenic bacterial strains. *Afr. J. Biotechnol.* 2010, *9*, 518–522.
59. Vardar-Unlu, G.; Candan, F.; Sokemen, Daferra, D.; Pollissiou, M.; Sokemen, M. Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Thymus pectinatus*. *J. Agri. Food Chem*., 2003, *51*, 63–67.
60. Vasanthi P, Venkatalakshmi S. Comparative study on the effect of Indian and gross bred cow urine distillate on the growth & food utilization parameter of *Cirrhinus Mrigala*. *Species*, 2015, 13(41), 79-88
61. Venkatesh, R.; Shanthi, S.; Rajapandian, K.; Elamathi, S.; Thenmozhi, S.; Radha, N. Preliminary study on antixanthomonas activity, phytochemical analysis and characterization of antimicrobial compounds from *Kappaphycus alvarezii*. *Asian J. Pharmaceut. Clin. Res*. 2011, *4*, 46–51.
62. Vinotha, M.; Thavasuraj, S.; Chinniah, S.; Nithya, V. Antimicrobial, antibiofilm and antioxidant effects of medicinal plants extract with indigenous cow ark against human pathogens. *Int. J. Adv. Sci. Technol*. 2020, *29*, 569–583.
63. Wate, S.P.; Dhanjode, D.P.; Duragkar, N.J.; Tajne, M.R. Antioxidant potential of cow urine and its fractions: A




ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

comparative study. *Int. J. Univ. Pharm. Life Sci*. 2011, *1*, 146–54.

64. Yadav, B.K.; Lourduraj, A.C. Use of Panchagavya as a growth stimulant and biopesticide in agriculture. In: *Environment and Agriculture*, Kumar, A. Ed., APH Publishing Corporation, New Delhi, 2005, pp 65–70.
65. Yadav, S.J.; Thakare, S.B. Cow dung for improving the pH of highly alkaline soil and Indian cow importance from vedic scriptures. *Int. J. Sci. Res.*, 2013, *4*, 1559–1562.



ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS