International Journal of Current Research in Multidisciplinary (IJCRM) *ISSN: 2456-0979*
Vol. 7, No. 12, (December'22), pp. 06-10

COMPARATIVE ANALYTICAL AND ACTIVITY PROFILE OF *GOMUTRA ARKA* (COW URINE) COLLECTED IN EARLY MORNING AND EVENING HOURS OF A DAY WSR TO ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT: Gomutra (Cow urine) has lot of medicinal application in Ayurveda. Being potential source of medicine used as antimicrobial, anticancerous, medhya, antihyperlipidemic, hepatoprotective etc. Ayurveda advocates to collect cows urine after Jeernahara(after complete digestion of food) ie early morning as these are cud chewing animals. Hence with all this background a study has been designed to evaluate the Gomutraarka (Cow urine distillate) collected at early morning and evening hours of a day wsr to analytical and antimicrobial study.
KEYWORDS: Gomutra (Cow urine), arka, analytical, antimicrobial

INTRODUCTION

Cow (Bos indicus) urine/Gomutra has a long history of therapeutic use in India. Ayurveda describes Gomutra as the best of all types of animal urine (including human) and enumerates its various therapeutic uses. Gomutra is called nectar when taken properly[1]. In addition, it has applications as a biopesticide in organic farming along with cow dung, cow's milk and other herbal ingredients[2].

Chemically cow urine is comprising of 95% water, 5% urea, minerals, salts, hormones and enzymes, which include iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, sulphur, phosphates, potassium, amino acids, enzymes, cytokine and lactose[3]. Since it is rich in these essential minerals, capable of removing many imbalances in the human body, thus maintaining general health. Lots of researches have been conducted in Cow Urine Treatment. Gomutra is said to be capable of curing blood pressure, blockage in arteries, arthritis, diabetes, heart attack, cancer, thyroid, asthma, psoriasis, eczema, prostate, fits, AIDS, piles, migraine, ulcer, acidity, constipation, gynecological problems and several other diseases[4]. Pharmacologically it is said to be antimicrobial, anticancer, antioxidant, immunostimulant, immunomodulatory, bioenhancer [4].

In Ayurveda, arka can be defined as a liquid preparation obtained by distillation of certain liquids or drugs soaked in water using the Arka-yantra or any convenient modern distillation apparatus [5]. The distillate of cow urine is called GomutraArka. It has a longer shelf life than cow urine and edible. Properties are said to be similar as that of urine [6].

Ayurveda advocates to collect cows urine after Jeernahara(after complete digestion of food) ie early morning as these are cud chewing animals[7]. But commercially urine is collected at different hours of the day. Hence with all this background a study has been designed to evaluate the Gomutraarka (Cow urine distillate) prepared out of cow urine, collected at early morning and evening hours of a day, to evaluate comparative analytical and activity profile wsr to its antimicrobial activity.

MATERIALS AND METHODS

2.1 Materials:

2.2 Preparation of Gomutraarka(Cow urine distillate):

Cow urine (Bos indicus)(indigenous variety) reared under natural condition was collected at early morning (6 am)(sample A) and at evening (6 pm) (sample B).Both urine samples were collected and arka is prepared separately using Arka yantra. The arka prepared was collected and stored in air tight bottles. And labelled as S1(Morning sample) and S2(evening sample). Around 600ml of arkawas collected from 1 liter of Gomutra. Both samples of Arka S1 and S2 subjected for analytical and antimicrobial study as per standard methodology.

2.3 Methodology:

2.4 Analytical study[8]:

Specific gravity Cleaned a specific gravity bottle by shaking with acetone and then with ether. Dried the bottle and noted the weight. Cooled the sample solution to room Temperature. Carefully filled the specific gravity bottle with the test liquid, inserted the stopper and removed the surplus liquid. Noted the weight. Repeated the procedure using distilled water in place of sample solution.

Refractive index

Placed a drop of water on the prism and adjusted the drive knob in such a way that the boundary line intersects the separatrix exactly at the center. Noted the reading. Distilled water has a refractive index of 1.3315 at 34˚C. The difference between the reading and 1.33144 gives the error of the instrument. If the reading is less than 1.3315, the error is minus (-) then the correction is plus (+)if the reading is more, the error is plus (+) and the correction is minus (-). Refractive index of oil is determined using 1 drop of the sample. The correction if any should be applied to the measuredreading to get the accurate refractive index. Refractive index of the test samples were measured at 28˚C.

Viscosity

The given sample is filled in a U tube viscometer in accordance with the expected viscosity of the liquid so that the fluid level stands within 0.2 mm of the filling mark of the viscometer when the capillary is vertical and the specified temperature is attained by the test liquid. The liquid is sucked or blown to the specified height of the viscometer and the time taken for the sample to pass the two marks is measured. Viscosity is measured using the formula

$\eta 1 = (\rho 1 t 1 \pm \eta 2)/\rho 2 t 2$

$\eta 1$ – Visosity of sample

$\eta 2$ - Visocity of water

t1 and t 2- Time taken for the sample and water to pass the meniscus

$\rho 1$ and $\rho 2$ – Density of sample and water

Determination of pH

Preparation of buffer solutions:

Standard buffer solution: Dissolved one tablet of pH 4, 7 and 9.2 in 100 ml of distilled water. Determination of pH: 1 ml of sample was taken and make up to 10 ml with distilled water, stirred well and filtered. The filtrate was used for the experiment. Instrument was switched on. 30 minutes time was given for warming pH meter. The pH 4 solution was first introduced and the pH adjusted by using the knob to 4.02 for room temperature 30°C. The pH 7 solution was introduced and the pH meter adjusted to 7 by using the knob. Introduced the pH 9.2 solution and checked the pH reading without adjusting the knob. Then the sample solution (S1 and S2) introduced and reading were noted. Repeated the test four times and the average reading were taken as result.

2.5 In-vitro antimicrobial study[9]:

The in-vitro antimicrobial study will be performed using well diffusion method. Agar media is inoculated with loop of desired microorganism (Table 1). Test will be conducted for different concentrations of extract at 10, 25, 50, 100 ml and kept for incubation. The presence of zone of inhibition will be observed on Petri dish with respect to standard drug. Both samples S1 and S2 are used for comparative in-vitro antimicrobial study.

2.6 Preparation of Nutrient agar media:

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally, 15 g agar was added to the media and autoclaved at 121˚C for 20 minutes.

2.7 Preparation of the inoculum[10]:

Micro-organism procured from Microbial Type Culture Collection and Gene Bank (MTCC), IMTECH, Chandigarh. (Table 1) Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

2.8 Well diffusion method[11]:

The media was cooled to around 45-55˚C, around 20ml each was poured into sterile petri plates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37˚C and observed after 24 h.

RESULT

Table 1. Analytical parameters of Gomutra Arka:

Parameter	**Results n=3 percentage w/w**	
	S1	**S2**
Specific gravity	1.0052	1.0033
Refractive index	1.33344	1.33194
Viscosity	0.9768	0.9751
PH	7.0	7.0

In vitro antibacterial activity:

Table 2. Microorganism strains used

Microorganism strain	Code	Standard drug used
Staphylococcus aureus	MTCC 3160	Ampicillin
Streptococcus pyogens	MTCC-86	Ampicillin
Candida albicans	MTCC-183	Clotrimazole

Table 3: In vitro antibacterial activity of S1 (M) and S2 (E) samples against S. aureus.

Sample	Volume	Zone of inhibition – (Radius in mm)	
		S1 (M)	**S2 (E)**
S1(M)	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	0	0
Standard (Ampicillin) 1 mg/ml	30 µl	12	12

Table 4: In vitro antibacterial activity of S1 (M) and S2 (E) samples against *Streptococcus pyogens*

Sample	**Volume**	**Zone of inhibition – (Radius in mm)**	
		S1 (M)	**S2 (E)**
S1(M)	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	2	0
Standard (Gentamicin 240 µg)	10 µl	15	15

Table 5: In vitro antifungal activity of S1 (M) and S2 (E) samples against *Candida albicans.*

Sample	Volume	Zone of inhibition – (Radius in mm)	
		S1 (M)	S2 (E)
S1(M)	25 µl	0	0
	50 µl	0	0
	75 µl	0	0
	100 µl	4	0
Standard (Clotrimazole)	10 µl	09	09

DISCUSSION

Cow (*Bos indicus*) urine/*gomutra* used since centuries in different pathological conditions in Ayurveda. Researches carried out on this also suggest it as antimicrobial, anticancer, bioenhancer, immune booster. Classical texts of Ayurveda advocates to collect Mutra(urine) after Jeernahara(after complete digestion) ie early morning. Hence with all these backgrounds a study has been planned to conduct comparative analytical and antimicrobial property of Gomutraarka which is prepared using early morning sample(S1) and evening sample(S2) from naturally reared cows.

Analytical parameters like specific gravity, Refractive index, Viscosity and PH of S1 and S2 were carried out as per standard protocol. Specific gravity of S1 was 1.0052 whereas that of S2(evening sample was 1.0033). Morning sample (S1) specific gravity was much higher than that of evening collected sample. PH of both samples shown similar value. Refractive index denotes the density of liquid. S1(morning sample) had shown higher value of Refractive index (1.33344) than that of S2(evening sample). Similarly, morning sample found more viscous than that of evening sample.

In vitro Antimicrobial study was conducted on three microbial pathogens ie*Streptococcus pyogens*, *Staphylococcus aureus* and *Candida albicans*. Comparative antimicrobial study of both samples on these three pathogens has not shown much difference. Both S1 (M) and S2 (E) sample not showed antibacterial activity against S. aureus. S1 (M) has shown zone of inhibition of 2mm at 100µl and S2 (E) sample not showed activity against *Streptococcus pyogens.* S1 (M) has shown zone of inhibition of 4mm at 100µl and S2 (E) sample not showed antifungal activity against *Candida albicans.* Thus, in total morning sample has shown slight antibacterial and antifungal activity, whereas S2(E) has not shown any activity. With this we can confirm the classical reference as morning collected cow urine will be better than that of evening collection.

CONCLUSION

Ayurveda science of life has its scientific guidelines for drug collection of plant, animal, mineral origin. Gomutra(Cow urine) being potential source of medicine used as antimicrobial, anticancerous, medhya, antihyperlipidemic, hepatoprotective. Ayurveda advocates to collect cow urine after Jeernahara(after complete digestion of food) ie early morning as these are cud chewing animals. In this study S1(M) morning sample has shown slight antibacterial and antifungal activity, whereas S2(E) has not shown any activity. With this we can confirm the classical reference as morning collected cow urine will be better than that of evening collection.

ACKNOWLEDGEMENT

Part of the observations is made from findings of a student research project entitled “Comparative analytical and activity profile of Gomutra-arka collected in early morning and evening hours of a day with respect to its antimicrobial activity”RGU/ADV_RES/UG-RESEARCH/98/2020-21 dated18/02/2021, sanctioned by Rajiv Gandhi University of Health Sciences, Bangalore.

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