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Research Article

Study of the Antimicrobial activity of Cow urine and Medicinal plant extracts on Pathogenic Human Microbial strains

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ABSTRACT

Since last few decades, medicinal treatment to counter microbial infections has been pivoted on synthetic drugs based antibodies and chemotherapeutic agents. The greatest disadvantage of these products is the resistance developed by the host-body against their action, finally rendering them ineffective to counter the disease, and in addition, the host faces innumerable adverse reactions of these drugs. These reactions are not observed from drugs developed from natural products. Hence, research has been diverted in this aspect since last few years. The current research is focussed to evaluate the comparative invitro antimicrobial activity of Cow urine with medicinal plant extracts against common pathogenic microbial strains. The work is focussed into the detail study of the antimicrobial potential of: (1) Fresh Cow urine, (2) Photo Activated Cow Urine, (3) Cow Urine Distillate and (4) Sterile Cow Urine; on pathogens like: *Bacillus subtilis* (NCIM 2063), *Escherichia coli* (NCIM 5051), *Proteus vulgaris* (NCIM 2027), *Salmonella typhi* (NCIM 2501) and *Staphylococcus aureus* (NCIM 2127), The medicinal plant extracts selected are: *Asparagus racemosus, Ocimum sanctum, Tinospora cordifolia* and *Withania sominifera*. The antibacterial activity was assessed by agar well diffusion technique. Test results indicated the prominent effectiveness of Photo Activated Cow Urine and Sterile Cow Urine to counter the pathogens. All four cow urine samples promoted best antimicrobial activity against *Salmonella typhi* and *Bacillus subtilis*. Satisfactory antimicrobial activity of medicinal plant extracts prepared in alcohol, methanol, and water was observed. Suppression of the growth of *Proteus vulgaris* was highest by the different plant extracts, whereas in case of *Salmonella typhi* it was the least. The methanol and acetone extracts of all the plants showed better antimicrobial effect than the aqueous extracts. This compilation of the results indicates that cow urine and medicinal plant extracts exhibit better antimicrobial action against different clinical microbial strains; hence can be utilised to control microbial infections. Still it is a need of the day to assess the same effect in, in vivo and to examine the complied effect of cow urine and medicinal plant extracts on pathogenic microbial strains.

Key words: Antibacterial activity, Gomutra therapy, medicinal plant extracts, pathogenic bacteria.

INTRODUCTION

Microorganisms like bacteria and fungi are mainly responsible for the increased infections in human beings and also animals, as these spore formers create problems in treatment. These organisms possess the genetic ability to gain resistance towards synthetic chemotherapeutic drugs, and on the contrary, the host experiences many adverse effects of these drugs.

Hence to rectify such abnormal and grave situation, researchers are focussing towards finding naturally available products. Nature is an abundant first rate

store-house consisting of an enormous range of plants, animals and microorganisms available for the discovery and further development and supply of these products, which are capable to counter almost all microbial infections prevalent in humans. The Indian Cow, *Bos indicus*, is a most veneered and valuable animal in religious scriptures. Cow urine has found therapeutic applications since days of yore. Cow urine is consumed by the majority of the rural population as a traditional remedy in almost the whole Indian continent[1].

Cow urine based preparations are able to counter viral, microbial, and fungal ailments. These potions promote powerful antimicrobial, antiviral, anti allergic, and antioxidant activity. So the current research is mainly centred on the exploration of the antimicrobial powers of cow urine and also its phyto chemical properties[3].

India has a large geographical area with different cultural practices that employ medicinal plants; the knowledge being recorded in medicinal texts and manuscripts in the form of *Samhitas* and varying regional practices to be available for the generations to follow[4]. The Indian *Ayurveda* is the result of this codified system of medicine developed through ages, documenting over 25000 herbs. The positive effect of these plants is of scientific interest for researchers in and around the world. So, in order to further strengthen this activity, the current research has been centred to explore the antimicrobial, antifungal, and immune modulator activity of the selected widely available medicinal plants.

MATERIALS AND METHODS

Procurement of Cow Urine:

Cow urine was collected from the well-maintained *Goshala* of Gurukulsupa near Navsari which has forty different breeds of cow. The cow selected for this research was a healthy Gir cow, aged seven years being fed a uniform diet and undergoing regular vaccination schedule.

Fresh cow urine was collected in sterile screw cap bottles and brought to the laboratory for testing. It was filtered by ordinary filter paper before being subjected to further testing. Sterile cow urine was prepared by sterilizing the urine sample maintained in an autoclave at a temperature of 121°C and 15 lb/in^2 pressure for 15 minutes. Photo activated cow urine was prepared by keeping the urine in transparent sterile bottle for 144 hours in sunlight. Then it was purified on a silica gel G-25 column and passed through two separate columns simultaneously to get rid of all the precipitated materials and debris[5]. Cow urine distillate was obtained by subjecting the fresh urine sample to a distillation unit where the distillate is obtained after 4 – 5 hours of distillation. Thus purified cow urine and/or its distillate were stored at 4 °C for further future use as per experimental requirements [6, 7, 8, 9].

Different samples of cow urine thus obtained are:

- Fresh cow urine,
- Sterile cow urine,
- Photo activated cow urine,
- Cow urine distillate, were then used for the study.

Plant Material:

Medicinal plants used in the study were collected from the botanical section of Navsari Agriculture University. The plants were subjected to different processes to prepare their extracts.

The plants were properly washed with water, air-dried, and then finely ground and then mixed separately with Water, Acetone and Methanol. Three separate solvent extractions using Aqua (Water), Acetone and Methanol were carried out using a Soxhlet extractor[10].

100 grams of dried leaves were powdered and then extracted with 200 ml of the solvent. The extracts were then filtered using Whattman filter paper No. 42 (125 mm), and then stored in air tight dark bottles at room temperature for assessing their antimicrobial activity[11, 12].

The plants selected in the study are:

1) *Asparagus racemosus*, locale name: *Satavari*,
2) *Ocimum sanctum*, locale name: *Tulsi.*
3) *Tinospora cordifolia*, locale name: *Guduchi*,
4) *Withania Sominifera*, locale name: *Ashwagandha*.

Fungal and Bacterial Cultures:

Standard fungal and microbial cultures were sourced from: National Collection of Industrial Microorganisms (NCIM), Pune.

The organisms under study are:

1) *Aspergillus fumigatus* (NCIM 902),
2) *Bacillus subtilis* (NCIM 2063),
3) *Candida albicans* (NCIM 3471).
4) *Escherichia coli* (NCIM 5051),
5) *Proteus vulgaris* (NCIM 2027),
6) *Salmonella typhimurium* (NCIM 2501),
7) *Staphylococcus aureus* (NCIM 2127),

The cultures were allowed to grow on their respective selective media to check and ensure their purity and optimum growth before subjected to futher tests.

Table 1
Antimicrobial activity of different samples of cow urine.

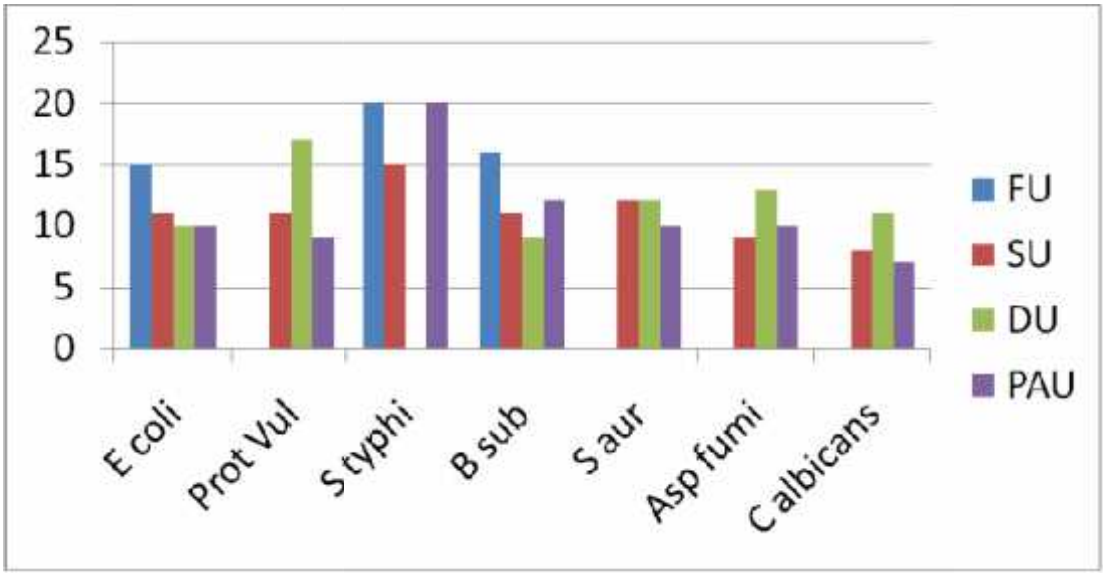
Name of organism	FU	SU	DU	P AU
Escherichia coli	15	11	10	10
Proteus vulgaris	--	11	17	09
Salmonella typhi	20	15	--	20
Bacillus subtilis	16	11	09	12
Staphylococcus aureus	--	12	12	10
Aspergillus fumigatus	--	09	13	10
Candida albicans	--	08	11	07

Zone of inhibition, in mm.

Table 2
Antimicrobial activity of methanol, acetone and aqueous extracts of medicinal plants.

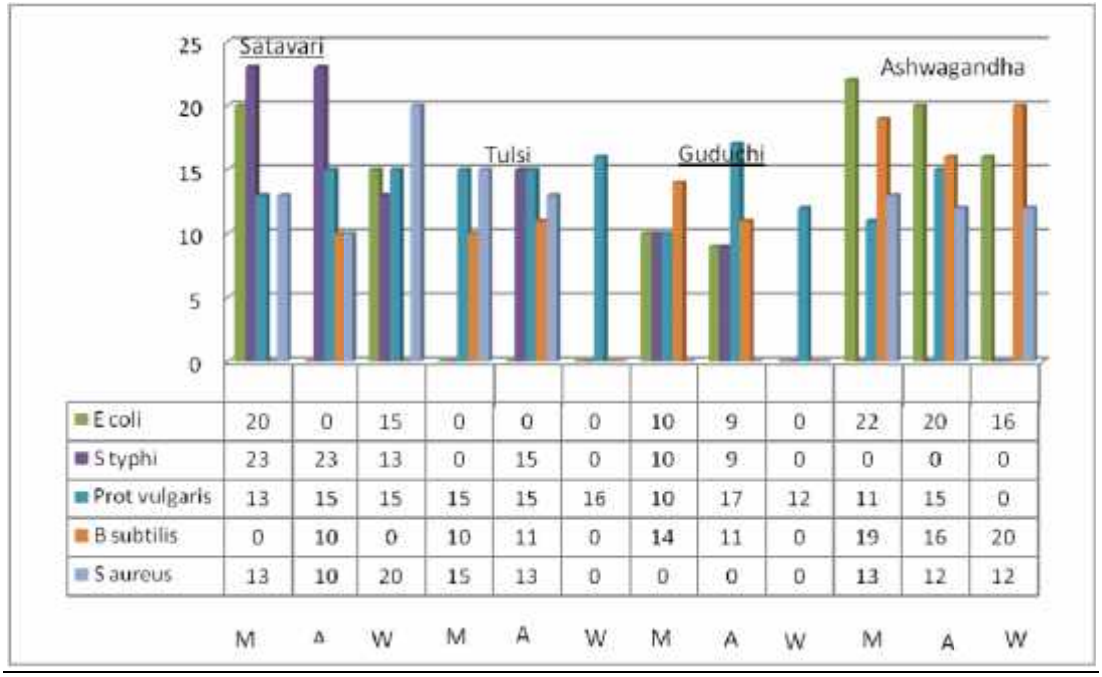
Name of organism	SATAVARI			TULSI			GUDUCHI			ASHWAGANDHA		
	M	A	W	M	A	W	M	A	W	M	A	W
E. coli	20	--	15	--	--	--	10	09	--	22	20	16
S. typhi	23	23	13	--	15	--	10	09	--	--	--	--
Prot. vulgaris	13	15	15	15	15	16	10	17	12	11	15	--
B. subtilis	--	10	--	10	11	--	14	11	--	19	16	20
S. aureus	13	10	20	15	13	--	--	--	--	13	12	12

Signages: A: Acetone Extract; M: Methanol Extract; W: Aqueous Extract; Zone of inhibition, in mm.



Signages: FU: Fresh Urine; SU: Sterile Urine; DU: Distilled Urine; PAU: Photo Activated Urine

Fig 1
Antimicrobial activity of different samples of cow urine



E coli	20	0	15	0	0	0	10	9	0	22	20	16
S typhi	23	23	13	0	15	0	10	9	0	0	0	0
Prot vulgaris	13	15	15	15	15	16	10	17	12	11	15	0
B subtilis	0	10	0	10	11	0	14	11	0	19	16	20
S aureus	13	10	20	15	13	0	0	0	0	13	12	12

Fig 1
Antimicrobial activity of methanol, acetone and aqueous extracts of medicinal plants.

Assay Medium:
Mueller-Hinton agar medium (Hi Media) is selected to check the potency of the drug against different microorganisms under study. Kirby-Bauer technique was employed to assess the antimicrobial potential of cow urine samples and medicinal plant extracts[13].
Antimicrobial Activity
Antimicrobial activity test was conducted by agar well diffusion method according to the following steps:

- 20 ml of sterile Mueller-Hinton agar was poured in sterile petri dishes.
- The plates were allowed to solidify before use.
- The organisms (0.2 ml) were uniformly spread onto the agar plate using a spreader.
- 8 mm bores were made, each at an equal distance from one another on the medium with a sterile cup borer.
- 20 µl of different cow urine preparations and plant extract preparations were added to respective bores.
- The plates were incubated at 37 °C for 24 hours, and the zone of inhibition was measured[14].

RESULTS AND DISCUSSION
Antimicrobial potential of different cow urine preparations are shown in Table 1 and Figure 1.
It is deduced from the current study that both, sterile urine and photo activated urine were most effective against all the different test organisms under study. Effectiveness of Fresh cow urine was observed to be nil against *Asperigillus fumigates*, *Candida albicans*, *Proteus vulgaris* and *Staphylococcus aureus*. Cow Urine Distillate exhibited maximum growth suppression in *Asperigillus fumigatus*, followed by *Candida albicans*. Amongst all the different cow urine samples, highest antimicrobial activity was observed against *Salmonella typhi*, followed *by Bacillus subtilis*.
Antimicrobial properties of Fresh Cow Urine were very low, but after photo-activation, it proved to be very effective in controlling the proliferation of the microorganisms. This increased action may be due to the hydrolytic state of cow urine and the presence of amino acids in urinary peptides, by increasing the bacterial cell surface hydrophobicity. Further increase in the antimicrobial activity of cow urine may be due to the formation of reactive compounds like formaldehyde, sulffinol, ketones, and amines during long term storage, heating, and photo activation.
From Table 2 and Figure 2 it is deduced that the different extracts of medicinal plants showed antimicrobial activity against different test organisms in varying proportions. Growth of *Proteus vulgaris* was more suppressed by the different plant extracts, and *Salmonella typhi* promoted the least growth suppression. The plant extracts prepared in acetone and methanol showed better antimicrobial action than the aqueous extract.

Ocimum sanctum and *Withania Sominifera* showed good antimicrobial activity against all test organisms followed by *Tinospora cordifolia* which exhibited least effect; while *Asparagus racemosus* was totally ineffective against all the microorganisms.

CONCLUSION

Phyto chemical investigation of cow urine sample and medicinal plant extracts will definitely prove the presence of active phyto constituents like alkaloids, anthraquinones, flavonoids, tannins and saponins; which are the main constituents promoting antimicrobial activity. Further research in this direction will help to develop proper formulations of cow urine and medicinal plant extracts to counter various microbial infections, thus boosting the human immune system.

REFERENCES

1. Jerald E, and Edwin S, *et.al.,* Antioxidant and antimicrobial activities of cow urine; Global Journal of Medicinal Plant Research, 2008; 5(4): 649-651.
2. Dr. Piyush Mishra, (2005), Article about Bhaktivedanta Swami Goshala (Iskon Vrindavan); Explorer: Article
3. Shivkumar, Dineshkumar Singh, and Vinaykumar Singh. Molluscicidal effect of freeze dried cow urine of Indian cow breeds against Lymnaea acuminate; American Eurasian Jr. of Toxocological sciences 2011; 3(2):70–74.
4. Lakshmi Chandra Mishra, (2004); Book: Scientific basis for Ayurvedic Therapies, CRC press Boca Raton, London. New York. Washington DC.
5. Sathasivan A, *et.al.,* Antimicrobial activities of cow urine distillate against some Clinical pathogens; Global journal of Pharmacology 2010; 4(1): 41-44.
6. Mandavgane SA, Rambhai AK, and Mude NK. Development of cow urine based disinfectant; Pharmacology. 2005; 2(2): 20-22.
7. Randhwa GK. Cow urine distillate as bio enhancer; Journal of AIM. 2010;1(4): 240-241.
8. Upadhyay R, Dwivedi P and Ahmed S. Antimocrobial activity of photoactivated cow urine against certain pathogenic bacterial strains; African journal of biotechnology 2010; 9(4): 518-522.
9. Gurpreet Kaur Randhawa. Cow Urine Distillate as Bioenhancer; J Ayurveda Integr Med., 2010; 1(4): 240-241.
10. Aibinu *et.al.* Invitro antimicrobial extracts from plants Bryophillum pinnalum & Kalanchoe crenata , Afr Jr of Trad CAM. 2007; 4(3):338-344.
11. Ganguly S, and Arun Prasad. Role of plant extracts and cow urine distillate as immunomodulators; International Journal of Pharmaceutical Sciences and Research 2011; 2(7): 1781-1785.
12. Kamba–Hasan. Phytochemical screening & antimicrobial activites of Euphorbia balsamifera leaves, stem and roots against some pathogenic micro organisms; African Journal of Pharmacy & Pharmacology. 2010; 4(9):645-652.
13. Bauer AW, Kirby WM, Sherris JC, and Turck M. Antibiotic susceptibility testing by standardised single disc or cup method. Am Jr Clin Pathology.1966; 45:493-496.
14. Gerard J. Tortora, *et.al.,* Microbiology, An Introduction, 4th edition.