

ANTIMICROBIAL ACTIVITY OF PANCHACAVYA AGAINST URINARY TRACT INFECTION

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ABSTRACT

Objective: The present study analyzes the antibacterial activity of the natural product. Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow namely Urine, Dung, Milk, Ghee Curd and the natural product of honey.

Methods: The sample used as a different concentration of agar well diffusion technique.

Results: Among the natural products, honey/100 µl given the maximum zone of inhibition against *Staphylococcus aureus* (33 mm), *Pseudomonas spp.* (31 mm), followed by *Klebsiella spp.* (28 mm) and *Escherichia coli* (28 mm). The crude extract of *Lactobacillus sp.* (buttermilk and curd) showed the considerable zone of inhibition in all the four isolates such as *Pseudomonas spp.*, *Klebsiella spp.* followed by *Staphylococcus aureus* and *Escherichia coli*. There is a considerable zone of inhibition for cow dung was observed only in *Staphylococcus aureus*. Among the natural products honey, milk, buttermilk was effective.

Conclusion: All urine samples were aseptically inoculated on Eosine Methylene Blue agar (EMB), MacConkey Agar and Blood agar and incubated.

Keywords: Panchakavya, Antibacterial activity, Agar well diffusion agar

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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. Urinary tract infection is one of the most common infectious diseases which have been extremely studied in the field of clinical practice [1]. Different products obtained from cow milk, ghee, curd, urine, and dung are used widely in a number of Ayurvedic formulations. Cow dung is traditionally used as organic fertilizer in Indian sub-continental farming for centuries [2]. Cow's urine having curative properties in skin diseases, especially leprosy is referred to in *Caraka samhita*. Indian cow urine is very powerful medicine in the Ayurvedic side. 'The cow' is a mobile medical dispensary and cow urine is a panacea of all diseases. The cow urine, one of the ingredients of 'Panchagavya' is capable of treating many curable as well as incurable diseases and has been used extensively in Ayurvedic preparations since time immemorial as cited in ancient holy texts like Charaka Samhita, Sushruta Samhita, Vridhabhagabhatt, Atharva Veda, Bhavaprakash, Rajni Ghunt, Amritasagar, etc [3]. Honey has a long medicinal history. The ancient Egyptians made offerings of honey to their gods used it as an embalming fluid and dressing for wounds. Today, many people swarm to honey for its antibacterial and anti-inflammatory properties. Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. The use of honey as a traditional remedy for microbial infections dates back to ancient times. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature [4]. Honey has previously been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram-positives, and Gram-negatives [5]. There are many reports of bactericidal as well as the bacteriostatic activity of honey and the antibacterial properties of honey may be particularly useful against bacteria, which have developed resistance to many antibiotics. Several properties in honey contribute to its antimicrobial activity. High osmolality, low pH, and hydrogen peroxide are the main antimicrobial factors. Also, phenolic compounds may contribute to the antimicrobial activity.

Medicinal property

Honey is an ancient remedy for the treatment of infected wounds, which has recently been 'rediscovered' by the Medical profession, particularly where conventional modern therapeutic agents fail. The first written reference to honey, a Sumerian tablet writing, dating back to 2100-2000 BC, mentions honey's use as a drug and an ointment. Honey has been reported to exhibit antimicrobial activity against pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) making this honey a promising functional food for the treatment of wounds or stomach ulcers. The honey has been used from ancient times as a method of accelerating wound healing, and the potential of honey to assist with wound healing has repeatedly been demonstrated [6]. The honey, when applied topically, rapidly clears wound infection to facilitate healing of deep surgical wounds with infection.

MATERIALS AND METHODS

Sample collection

Ten urine samples from Urinary tract infected patients were collected in Salem district during Dec, 2014 to Jan, 2015. The urine sample was brought to the laboratory in a sterile container within one hour after the collection and processed immediately.

Processing of honey for antibacterial activity

The honey sample was filtered through a sterile cheese cloth to remove debris, autoclaved at 121 °C for 15 min, streaked blood agar and nutrient agar plates in duplicate, and incubated for 24 h at 35 °C to check microbial purity. Different concentrations of each honey are constituting, 10, 20, 40, 60 and 80% (v/v) were made using sterile distilled water. This was done by dissolving the respective volumes: 1, 2, 4, 6, 8 ml of each honey into corresponding volumes of sterile distilled water to give a 10 ml preparation. The pH of the honey was checked and stored at 4 °C until used [7].

Processes of cow urine sample

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./in² pressure for 15 min. Photo activated cow urine was prepared by keeping the urine in the transparent sterile bottle for 144 h in sunlight [8].

Antibiotic sensitivity test

The natural product was used to antibiotic susceptibility pattern of the isolates was studied by Kirby Bauer’s disc diffusion method. Both broad spectrum and narrow spectrum antibiotics were used. The antibiotics tested were Vancomycin (30 mcg), Ampicillin (10 mcg), Tetracycline (30 mcg) and chloramphenicol (10mcg).

Agar well diffusion technique

Antimicrobial activity of cow urine, cow dung, milk, butter milk, ghee, and honey was used against the isolated bacterial pathogens was performed by agar well diffusion method. Muller Hinton agar medium plates were prepared and sterilized. After solidification, bacterial cultures were swabbed on the medium and wells were made and photoactivated cow urine, milk, butter milk, ghee (100 µl). Purified honey was poured into each well (20 µl, 40 µl, 60 µl, 80 µl and 100 µl).

RESULTS AND DISCUSSION

Ten urine samples from Urinary tract infected patients were collected from the patients of Salem district during Dec, 2014 to Jan, 2015. Four isolates were identified; they were *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella spp.* and *Pseudomonas spp.* The

predominant among the isolates were *Escherichia coli* and *Staphylococcus aureus*. The result was correlated with the finding [9] this higher prevalence in *E. coli* may be due to the faecal contamination, the prediction of the organism from the toilets and the shortness of the female urethra. All the isolates were tested for their antibiotic susceptibility.

Antibacterial activity of cow urine, milk, ghee, buttermilk curd and honey for the bacterial isolates has been determined by agar well diffusion method and the sensitivity pattern. Among the natural products, honey/100 µl given the maximum zone of inhibition against *Staphylococcus aureus* (33 mm), *Pseudomonas spp.* (31 mm), followed by *Klebsiella spp.* (28 mm) and *Escherichia coli* (28 mm). Similar findings were observed [10]. Honey has acidic pH between 3.2 to 4.5 which is low to be inhibitory for many bacteria. The antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content along with its acidic properties of gluconic acid and antiseptic properties [11]. The curd and buttermilk are enriched with a lot amount of lactic acid bacteria. The presence of lactic acid bacteria also produces antimicrobial metabolites [12]. There is a considerable zone of inhibition for cow dung was observed only in *Staphylococcus aureus*.

There is absence zone of inhibition was observed in cow urine and ghee. Cow urine from different cows had a different level of antimicrobial properties. The difference in the level of antimicrobial properties of different cow urine may be because of difference in chemical composition of urine which may arise due to several reasons [13].

Table 1: Antibiotic sensitivity test

S. No.	Antibiotic disc	Symbol	<i>E. coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsillaspp</i>	<i>Pseudomonas spp</i>
1.	Chloramphenicol	C [30]	25 mm	28 mm	21 mm	12
2.	Tetracyclin	TE[10]	13 mm	21 mm	16 mm	-
3.	vancomycin	VA[30]	17 mm	15 mm	12 mm	-
4.	Ampicillin	Am	8 mm	10 mm	6 mm	-
5.	Methicillin	Me	-	-	-	-

Table 2: Antibacterial activity of honey against the uropathogens

Different concentration	<i>E. coil</i>	<i>Staphylococcus aureus</i>	<i>Pesodomonas sp.</i>	<i>Klebseilla sp.</i>
20 µ	16 mm	20 mm	15 mm	15 mm
40 µ	20 mm	25 mm	19 mm	19 mm
60 µ	22 mm	28 mm	21 mm	22 mm
80 µ	24 mm	30 mm	24 mm	25 mm
100 µ	28 mm	33 mm	31 mm	28 mm

Table 3: Antimicrobial activity of panchagavya against the uropathogens

Sample	<i>E. coil</i>	<i>Staphylococcus aureus</i>	<i>Pesodomonas sp.</i>	<i>Klebseilla sp.</i>
Dung	-	18 mm	-	17 mm
Butter milk	15 mm	17 mm	20 mm	18 mm
Curd	13 mm	15 mm	17 mm	20 mm
Urine	-	-	-	-
Ghee	-	-	-	-

CONCLUSION

Ten urine samples from Urinary tract infected patients were collected from the patients of Salem district Dec, 2014 to Jan, 2015. The urine sample was brought to the laboratory in a sterile container within one hour after the collection and processed immediately.

All urine samples were aseptically inoculated on Eosine Methylene Blue agar (EMB), MacConkey Agar and Blood agar and incubated. The morphological character was observed. These were purified by subculturing onto nutrient agar plates for further identification. After incubation, the plates were observed for growth and the isolated colonies were identified by morphological and biochemical characteristics. Isolated colonies were identified by Gram's staining, Motility, Indole, Methyl Red, VogesProskauer, Catalase, Oxidase,

Citrate, Urease and Triple Sugar Iron test. Antibiotic susceptibility pattern of the isolates was studied by Kirby bauer disc diffusion method. Both broad spectrum and narrow spectrum antibiotics were used. The antibiotics tested were Vancomycin (30 mcg), Ampicillin (10 mcg), Tetracycline (30 mcg) and chloramphenicol (10mcg). Most of the antibiotics usually used for the treatment of UTI are resistant. It is now necessary to develop new antimicrobials and therapeutic agents having high effectiveness with no side effects, easy availability and less expensive.

Natural products used for antibacterial activity were Cow urine, Ghee, curd, Buttermilk, milk, cow dung and Honey. Photoactivated cow urine was prepared by keeping the urine in the transparent sterile bottle for 144 h in sunlight. The crude extract of *Lactobacillus sp.* from curd and buttermilk was isolated and plated on MRS slants. Bacteriocin was produced, and bacteriocin assay was also

performed. Agar well diffusion technique was performed for all the bacterial isolates. Photo activated cow urine, milk, butter milk, ghee (100 µl). Purified honey was poured into each well (20 µl, 40 µl, 60 µl, 80 µl and 100 µl).

Among the natural products honey, milk, buttermilk was effective. In further studies the compounds of the natural product have to identify using HPLC

CONFLICT OF INTERESTS

Declare none

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