

Formulation and evaluation of antimicrobial gel of cow urine distillate

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Abstract: In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. The Indian Cow, *Bos indicus*, is a most venerated and valuable animal in religious scriptures. Cow urine has found therapeutic applications since days of yore. Cow urine based preparations have powerful antimicrobial, antiviral, anti allergic, and antioxidant activity. So the current research is mainly centered on the exploration of the antimicrobial powers of cow urine and incorporation of cow urine into gel formulation. The gels were prepared by using carbopol 940, propylene glycol, glycerin, triethanolamine, distilled water along with cow urine distillate and *Azadirachta indica* extract. The prepared gels were evaluated for physical appearance, spreadability, pH, Homogeneity and viscosity. The present study analyzes the antibacterial and antifungal activity of Cow Urine Distillate against the clinical pathogenic microorganisms such as *Bacillus subtilis* and *Staphalococcus aureus*. Maximum antibacterial activity was observed in *Bacillus subtilis* than *Staphalococcus aureus*. Antifungal activity of cow urine distillate was analysed against *Aspergillus niger*. The result revealed optimum characteristics features of gel formulation and confirm antimicrobial potential of prepared gel.

INTRODUCTION

Nowadays there are many people suffering from microbial and fungal infections. There are many microorganisms which cause number of infections and as they are present in spore form they create problems in treatment. These organisms undergo genetic changes i.e. mutation and gain resistance towards synthetic chemotherapeutic drugs and also these drugs show various adverse effects on the consumer. Hence to rectify such abnormal and grave situation researchers are focusing towards finding some natural products which can effectively replace to counter all microbial infections prevalent in human without showing side effects or fewer side effects than chemotherapeutics.

The Indian cow which is considered as mother or goddess is most valuable animal in religious scriptures. Cow urine has found therapeutic applications since days of yore. In Veda, cow's urine was compared with nectar. The therapeutic uses of cow urine have been mentioned in various literatures and Vedas of ancient period. Cow urine has a unique place in Ayurveda and has been described in *Sushruta Samhita* and *Ashtanga Sangrah* to be the most effective secretion of animal origin with innumerable therapeutic values. An exhaustive reference of use cow urine in curing various skin disease especially leprosy is referred to in *Charaka Samhita*.

In India, medicines based on herbal origin have been the basis of treatment and cure for various diseases. The Indian Cow, *Bos indicus*, is a most venerated and valuable animal in religious scriptures. Cow urine has found therapeutic applications since days of yore. Cow urine based preparations have powerful antimicrobial, antiviral, anti-allergic, and antioxidant activity. So the current research is mainly centered on the exploration of the antimicrobial powers of cow urine and incorporation of cow urine into gel formulation (Majali et al., 2015a; Majali et al., 2015b).

MATERIALS AND METHODS

Preparation of cow urine distillate

Cow urine was distilled at 100°C using distillation apparatus. The single distilled cow urine Furthermore; it was acidified by lowering the pH below 2.0 with the addition of 85% orthophosphoric acid. The cow urine was again distilled at 100°C using a distillation apparatus to remove ammonia. The distillate was stored in sterile glass flask at refrigerator (4°C).

Preparation of *Azadirachta indica* extract

100 g of *A.indica* coarse powder of leaves was extracted with water. The extract was collected, evaporated in a water bath at atmospheric pressure and the solvent was completely removed using vacuum and stored at 4°C for further use.

Preparation of gel

The propylene glycol, carboxyvinyl polymer (Carbopol 940), glycerin and distilled water were mixed uniformly by stirring, and triethanolamine was added to the mixture while continuing the stirring. (Debnath S.K.et al.2009) Now the cow urine distillate and prepared *Azadirachta indica* extract were added to this gel base and stirred continuously to obtain gel of required consistency, composition are shown in Table 1.

Evaluation of Gel

pH - The pH of the gel formulations was determined by using digital pH meter.

Appearance - The prepared gel bases were inspected visually for clarity, color and presence of any particles.

Homogeneity - All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Determination of odor - Determination of odor was done by mixing gel in water and taking the smell.

Viscosity measurement - Viscosities of the formulated gels were determined using Brookfield

viscometer spindle no. 7 and speed 60 rpm at 25°C, the corresponding dial reading on the viscometer were noted. (Misal G.,et al.2012)

Antimicrobial evaluation:

Antibacterial activity of prepared gel formulations (G3, G4) were determined by the cup plate method against the gram-positive organisms *Bacillus subtilis* and *Staphylococcus aureus*. The bacteria were sub-cultured on Nutrient Agar medium. The petridish were incubated at 37°C for 24h. Then the zone of inhibition of each cup was observed as result.

Antifungal activity of cow urine distillate (Kekuda T.R.et al.2008) was analysed against *Aspergillus niger* on sabouraud's dextrose agar media.

RESULTS

The prepared gels were evaluated for physical appearance, spreadability, pH, Homogeneity, viscosity and results are given in table 2.

The prepared gels were also evaluated for antibacterial and antifungal activity. The formulation G3 and G4 were selected for antimicrobial evaluation on the basis of better viscosity. The gel base is used as control, cow urine distillate and *Azadirachta indica* extract (2:1) used as a standard. The zone of inhibition were given in table 3 and figure 1 and 2.

Table 1. Composition of formulations.

| Sr. No | Formulation | Carbopol (% w/w) | Propylene Glycol (ml) | Glycerin (g) | Triethanolamine (g) | Cow urine distillate (ml) | <i>Azadirachta indica</i> extract (ml) |
|--------|-------------|------------------|-----------------------|--------------|---------------------|---------------------------|--|
| 1 | G1 | 0.5 | 0.3 | 5 | q.s * | 2 | 1 |
| 2 | G2 | 1 | 0.3 | 5 | q.s * | 2 | 1 |
| 3 | G3 | 1.5 | 0.3 | 5 | q.s * | 2 | 1 |
| 4 | G4 | 2.00 | 0.3 | 5 | q.s * | 2 | 1 |

* Quantity sufficient (q.s.) to neutralize gel base

Table 2: Values of evaluation parameters of developed gel formulations

| Formulation Code | pH | Viscosity(cp) | Homogeneity | Spreadability | Appearances |
|------------------|-----|---------------|-------------|----------------|-----------------------|
| G1 | 7.1 | 2.48 | Homogenous | Easy to spread | Clear and Transparent |
| G2 | 7.2 | 2.52 | | | |
| G3 | 7.4 | 2.61 | | | |
| G4 | 7.3 | 2.76 | | | |

Table 3: Antimicrobial activity (Zone of inhibition of prepared gel formulations)

| Microbial strain | Zone of inhibition (mm) | | | |
|------------------------------|-------------------------|----------|----------------|----------------|
| | Control | Standard | G3 Formulation | G4 Formulation |
| <i>Staphylococcus aureus</i> | 0 | 19 | 8 | 10 |
| <i>Bacillus subtilis</i> | 0 | 20 | 10 | 11 |
| <i>Aspergillus niger</i> | 0 | 16 | - | 15 |



Figure 1: Antibacterial activity a) *Bacillus subtilis* b) *Staphylococcus aureus*



Figure 2: Antifungal activity (*Aspergillus niger*)

DISCUSSION:

All formulations G1 to G4 showed clear and transparent appearance, easy spreadability and homogeneous nature. The pH was between 7.1 to 7.4 showed similar to that of skin pH. The formulation G3 and G4 showed better viscosity than that of G1 and G2 formulations.

The *Bacillus subtilis* showed greater zone of inhibition than *Staphylococcus aureus* for both G3 and G4 formulations. The zone of inhibition was greater for standard than formulation.

CONCLUSION:

Cow urine distillate was found to have significant antimicrobial properties and study reveals almost same results when applied as gel formulation. It is successfully incorporated in gel formulation and *Azadirachta indica* helps to enhance antimicrobial activity.

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